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Soybean (Glycine max) response to multiple, sublethal exposures of 2,4-D and dicamba from vegetative through reproductive growth

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Soybean (Glycine max) response to multiple, sublethal exposures of 2,4-D and dicamba from vegetative through reproductive growth

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This study was conducted to determine whether soybean productivity is affected by multiple, sublethal herbicide exposures. The effects of dicamba and 2,4-D on soybean (*Glycine max*) productivity was investigated at 17 site-years. Relative to a single exposure of dicamba at R1, an additional exposure at either V3 or R3 reduced yield up to 23%. Three or more applications did not further decrease yields relative to an R1&R3 exposure. For 2,4-D, a single application to V3, R1, R3, or R5 soybean did not affect grain yield. However, two exposures of 2,4-D occurring from V3 through R3 reduced yield 5 to 7%. Three or more applications of 2,4-D had no effect on yield relative to exposing soybean to 2,4-D twice between V3 and R3. Exposing soybean to multiple, sublethal rates of auxin herbicides can reduce yield relative to a single exposure and may be most deleterious from flowering to initial pod set.

DEDICATION

I would like to dedicate this work to my wife Andrea and son Knox, both of whom mean the world to me. Andrea, you have been beside me throughout my very long tenure in graduate school. You have always been encouraging when days are long and life is tough. I have enjoyed every moment we have been together and am excited to continue life with you and Knox.

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I would also like to say thank you to Dr. Darrin Dodds, Dr. Jason Krutz, and Dr. Dave Spencer who have guided me through this process. Each of you has assisted me more than you know in completing my degree. All three of you are great mentors and I am lucky to know every one of you. I have also known numerous graduate students during my time and have been influenced by every single one. One in particular, Dr. Ryan Edwards, became a very dear friend, answered all of my questions my first summer, and encouraged me to apply for graduate school. To him I say: Chuck, I have *finally* finished what I started all those years ago. The last person I would like to thank is my friend and colleague Beau Varner. Beau, I have enjoyed working with you for the past seven years. You have been encouraging throughout our time together, and I am going to miss you and everything we did daily for Dr. Reynolds's research program.

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CHAPTER I

RESPONSE OF SOYBEAN TO MULTIPLE, SUBLETHAL DICAMBA EXPOSURES OCCURRING FROM VEGETATIVE THROUGH REPRODUCTIVE GROWTH

Abstract

Off-target movement of herbicides has been more problematic in recent years and can reduce crop yields. This study was conducted to determine whether crop productivity is affected by multiple, sublethal herbicide exposures. The effect of dicamba exposure frequency and growth stage on non-dicamba soybean (*Glycine max*) growth, development, and grain yield were investigated near Fayetteville, Arkansas; Belleville, Illinois; Lafayette, Indiana; Stoneville and Starkville Mississippi; Columbia, Missouri; North Platte, Nebraska; and South Charleston, Ohio on soil textures ranging from sandy loam to silty clay loam. A single, sublethal application of dicamba applied to non-dicamba tolerant soybean at V3, R1, or R3 reduced yield 10 to 15%. A dicamba application at R5 had no effect on soybean grain yield. Multiple sublethal applications of dicamba applied at all possible combinations of V3, R1, R3, and R5 growth stages reduced soybean grain yield 7% to 37% compared to the soybean that received no dicamba application. In relation to a single exposure at R1, an additional exposure at either V3 or R3 reduced yield an additional 2 to 23%. Three or more sublethal applications of dicamba did not further decrease yield relative to soybean having been exposed to dicamba at R1&R3. Exposing non-dicamba soybean to multiple, sublethal rates of dicamba can reduce yield relative to a single exposure and may be most deleterious when exposure occurs from flowering to initial pod set.

Introduction

Given the development and proliferation of herbicide resistant weed species in the past decade, companies are turning to some of agriculture's oldest herbicides for weed control. Dicamba tolerant soybean is projected to be widely adopted in the United States (Mortensen et al. 2012). Recently, these systems have provided good broadleaf weed control, but extensive off-target movement (OTM) and sensitive vegetation injury has been reported (Bradley 2017; Mueller and Steckel 2021).

Over the past couple of years, sensitive vegetation, specifically soybean, has been subjected to OTM of herbicides (Bradley 2017; Dowell 2017; Hager 2017; Hartzler 2017; Zaccaro et al. 2020). Non-dicamba tolerant soybean grown in proximity to soybean receiving dicamba applications may be exposed to OTM multiple times during the growing season. The flowering stages of soybean have been found by previous research to be most sensitive with respect to yield loss when compared to vegetative or later reproductive stages (Wax et al. 1969; Auch and Arnold 1978; Griffin et al. 2013; Robinson et al. 2013; Solomon and Bradley 2014; Jones et al. 2019a). Scholtes et al. (2019) reported that non-dicamba soybean yield was most impacted by dicamba when exposure to dicamba occurred between V3 to R2 growth stages and least susceptible when exposure occurred from R5 to R6.5.

The severity of non-dicamba soybean injury from exposure to dicamba can range from mild to severe and can be influenced by many factors including herbicide drift rate, growth stage at time of exposure, and inherent susceptibility to the auxin herbicide. Sublethal rates of dicamba can cause alarming levels of visual injury (Wax et al. 1969; Kelley et al. 2005; Johnson et al. 2012; Egan et al. 2014; Scholtes et al. 2019). Dicamba exposure injury symptoms on soybean have been previously described as leaf cupping, stem epinasty, and swelling of the stem (Al-

Khatib and Peterson 1999; Andersen et al. 2004; Sciumbato et al. 2004; Jones et al. 2019b). Pod malformation also has been observed when low doses of dicamba are applied to soybean during reproductive stages (McCown et al. 2018). Though numerous studies have been conducted examining the effects of a single dicamba exposure on non-dicamba soybean, little to no research exists documenting the effects of multiple dicamba exposures. Therefore, the objective of this study is to determine whether multiple, sublethal applications of dicamba to non-dicamba tolerant soybean affects crop productivity over that of a single exposure.

Materials and Methods

Experiments were conducted from 2018 to 2021 at seven locations across the United States to assess the susceptibility of non-dicamba tolerant soybean to various exposure timings of dicamba. Soil textures, row spacing, planting date, and soybean cultivar varied by location and are given in Table 1.1. At each location, the experimental design was a randomized complete block with four replications and plots four rows wide. Treatments consisted of dicamba (Clarity®, BASF) application at 2.8 g ae ha^{-1} to non-dicamba tolerant soybean at every possible combination of the following growth stages: V3, R1, R3, and R5. Dicamba application to V3 soybean were made when three to four fully expanded trifoliates were present. Treatments applied to soybean in reproductive growth stages focused on three different timings including R1, (initial flowering or a flower located anywhere on the plant); R3, (initial pod set or a 3/16" long pod in the upper four nodes); and R5, (pod fill or visible seed in one of the pods of the upper four nodes). A non-exposure treatment was included at each growth stage resulting in several treatments receiving only a single V3, R1, R3, or R5 application. Applications were made using a CO_2 pressurized backpack sprayer with TTI nozzles (Teejet Spraying Systems, Wheaton IL) calibrated to deliver 140 L ha^{-1}. Data collection included visual evaluation of soybean injury, height, yield, and yield components including nodes plant^{-1}, nodes with a pod, pods plant^{-1}, seeds plant^{-1}, and grams 100 seed^{-1}. Visual injury and height data were collected at fourteen and twenty-eight days after each treatment as well as at soybean maturity. At maturity, yield components from ten plants in each plot were selected for evaluation prior to machine harvest. Yield was collected from the center two rows of each plot and moisture was corrected to 13% for yield calculations.

Analysis of variance was conducted using the GLIMMIX procedure in SAS 9.4 (SAS Institute Inc., Cary, NC). Data were analyzed as a mixed model with dicamba application as a fixed effect and replication, location, and year as random effects. Three predetermined comparisons were performed to meet the objectives of this experiment. First, all single application treatments of dicamba were compared to determine the growth stage(s) at which a single exposure of non-dicamba soybean to dicamba was most detrimental. Second, all double applications of dicamba were compared to the most detrimental single exposure of non-tolerant soybean to dicamba. Third, three or more dicamba applications to non-tolerant soybean were compared to the most yield-limiting single or double exposure. All three comparisons included a non-treated as the positive control, while investigations of multiple exposures also included the most injurious exposure at a lesser number of dicamba applications as the negative control. Degrees of freedom were calculated using the Kenward-Rogers method and least-square means were considered different at $\alpha \leq 0.05$ (Kenward and Rogers, 1997).

Results and Discussion

The primary hypothesis of this study was that multiple, sublethal exposures of non-dicamba tolerant soybean to dicamba would be more detrimental to soybean growth and development than a single exposure. A single, sublethal application of dicamba applied to soybean at V3, R1, or R3 reduced yield 10 to 15% (Table 1.2). A dicamba application at R5 had no effect on soybean grain yield. Multiple sublethal applications of dicamba applied at all possible combinations of V3, R1, R3, and R5 growth stages reduced soybean grain yield 7% to 37% compared to the non-treated (Tables 1.4 and 1.6). Contrary to our hypothesis, multiple exposures of soybean to dicamba were not always more deleterious than a single exposure. For example, only a double exposure occurring at V3&R1, V3&R3, or R1&R3 reduced soybean yield relative to a single exposure at R1. Moreover, three or more applications of dicamba never decreased yield relative to soybean having been exposed to dicamba at R1&R3.

Single Exposure

Exposure of soybean to dicamba from vegetative through early pod set decreases soybean grain yield by altering yield components. A single dicamba application of 2.8 g ae ha^{-1} at the V3, R1, or R3 growth stage reduced soybean yield 10 to 15% relative to the control (Table 1.2). Dicamba application at V3 decreased seeds $plant^{-1}$ but had no effect on nodes with a pod, pod number, or seeds pod^{-1} (Table 1.3). Exposing non-dicamba soybean to dicamba at R1 decreased the number of nodes producing pods, pod number, seeds pod^{-1}, and seeds $plant^{-1}$ from 9 to 25%. An application of dicamba at R3 reduced nodes with pods 11%, seeds $plant^{-1}$ 17% and seeds pod^{-1} 8%. Applying dicamba to non-dicamba tolerant soybean at R5 had no effect on grain yield or any evaluated yield component. These results are generally in agreement with existing literature.

Dicamba generally has an adverse effect on soybean grain yield when exposure occurs from early vegetation through early pod set. Dicamba application rates ranging from 5.6 to 17.5 g ae ha^{-1} decreased grain yield up to 36% when applied to V3 to R1 soybean (Wax et al. 1969; Auch and Arnold 1978; Al-Khatib and Peterson 1999; Kelley et al. 2005; Anderson et al. 2004; Johnson et al. 2012; Griffin et al. 2013; Scholtes et al. 2019). Dicamba applied at 8.8 g ae ha^{-1}

reduced yield up to 7% when applied to R3 soybean (Jones et al 2019b; Scholtes et al 2019). Dicamba's adverse effect on soybean grain yield when applied from flowering through early pod set is attributed to a decrease in pods per plant, seed number, and/or seed weight (Kelley et al. 2005; McCown et al 2018). Similar to our results, an 8.8 g ae ha^{-1} dicamba application to R5 soybean had no effect on yield (Jones et al 2019b; Scholtes et al 2019). Exposing non-dicamba tolerant soybean to simulated drift rates of dicamba from vegetative through late pod set will likely have an adverse effect on yield components and decrease the yield of most soybean varieties.

Multiple Exposures

Multiple applications of dicamba to non-tolerant soybean may exacerbate loss in productivity relative to a single exposure. Relative to a single application at R1, applying dicamba to V3&R1, V3&R3, or R1&R3 soybean reduced yield an additional 2% to 23% (Table 1.4). The decrease in grain yield due to a second exposure at V3 or R3 may be attributed to a decline in seed weight (Table 1.5). Applying dicamba to soybean at V3&R5, R1&R5, or R3&R5 had no adverse effect on yield or yield parameters relative to a single exposure at R1. Moreover, exposing soybean to sublethal rates of dicamba up to four times had no adverse on yield or yield parameters relative to a double exposure at R1&R3 (Table 1.6 and 1.7). Relative to a single exposure at R1, the most deleterious effect on yield occurs when soybean is exposed to dicamba at R1 and R3, that is, from initial flowering to beginning pod set.

Conclusion

This study was conducted to determine whether soybean growth and development is adversely affected by multiple, sublethal herbicide exposures. Relative to a single, sublethal application of dicamba at R1, an additional exposure at either V3 or R3 reduced soybean yield up to 23%. Three or more sublethal applications of dicamba never decreased yield relative to soybean having been exposed to dicamba at R1 and R3. Exposing non-dicamba soybean to multiple, sublethal rates of dicamba can reduce growth and development relative to a single exposure and may be most deleterious when exposures occur from flowering to initial pod set.

Table 1.1 State, year, latitude, longitude, soil texture, maturity group, variety, planting date, population, and row spacing of all locations for a soybean study examining the comparison between one, two, and three plus exposures of 2.8 g ae ha^{-1} dicamba at multiple growth stages.

State	Year	Latitude	Longitude	Soil	MG[a]	Variety	Planting Date	Seed $Hectare^{-1}$	Row Spacing (cm)
Arkansas	2018	36.0994	-94.179	silt loam	4.8	Credenz 4818 LL	June-4-2018	247105	91
	2019	36.0994	-94.179	silt loam	4.8	Credenz 4820 LL	April-8-2019	345947	91
Illinois	2018	38.5123	-89.8412	silt loam	4.1	Asgrow 4135	June-6-2018	345947	76
	2019	37.7955	-89.2589	silt loam	3.7	Pioneer P37T09L	May-25-2019	345947	76
Indiana	2018	40.2972	-86.9035	silt loam	3.0	Specialty 3005CR2	May-22-2018	345947	76
	2020	40.2972	-86.9035	silt loam	3.4	DynaGrow S34GL79	May-24-2020	345947	76
Missouri	2018	38.8978	-92.2192	silt loam	3.4	Pioneer P34TO7	May-18-2018	358302	76
	2019	38.8978	-92.2192	silt loam	4.6	Becks 465R4	May-17-2019	345947	76
Mississippi	2018	33.424	-90.9155	sandy loam	4.6	Asgrow 4632	April-4-2018	370658	102
	2018	33.424	-90.9155	sandy loam	4.6	Asgrow 4632	April-4-2018	370658	102
	2018	33.4743	-88.7716	sandy loam	4.6	Asgrow 4632	May-2-2018	321237	76
	2019	33.4743	-88.7716	sandy loam	5.1	Terral 51A56	May-17-2019	321237	76
	2020	33.4743	-88.7716	sandy loam	4.8	GoSoy 481E19	May-13-2020	345947	76
	2021	33.4743	-88.7716	sandy loam	4.6	Syngenta 46E3	May-20-2021	345947	76
Nebraska	2019	41.0899	-100.7675	silt loam	2.6	Asgrow 2636	May-17-2019	345947	76
Ohio	2018	39.5135	-83.401	silty clay loam	3.8	Credenz 3841 LL	June-6-2018	370658	76
	2019	39.8496	-83.6611	silty clay loam	3.3	Seed Consultants 3357LL	June-4-2019	358302	76
	2020	39.8609	-83.6722	silty clay loam	3.3	Seed Consultants 3319LL	May-13-2020	395368	76

[a] MG is an abbreviation for Maturity Group

Table 1.2 Grain yield, plant height at harvest, and visual plant injury at harvest in soybean from multiple locations and years across the U.S. receiving one dicamba exposure at 2.8 g ae ha^{-1} across multiple growth stages.

Main Effects	Yield	Plant Heights at Harvest[a]	Visual Injury at Harvest[b]
	kg ha^{-1}	cm	%
Non-treated	3944 a[c]	96 a	0 d
V3	3551 (10) b[d]	88 (8) b	4 c
R1	3385 (15) c	76 (21) c	20 a
R3	3520 (11) bc	79 (18) c	14 b
R5	3876 (2) a	95 (1) a	3 cd
		ANOVA	
		F Value and Significance Level	
Single Application	17.80***	37.00***	22.71***

[a] Heights recorded in centimeters from ground to apical meristem.
[b] Visual injury ratings rated on a scale of 0 to 100% with 0%=no injury and 100%=complete death.
[c] Within columns and by main effect, means followed by the same letter are not significantly different.
[d] Value in parentheses is percent reduction in relation to the non-treated.
*Significant at the 0.05 probability level. **Significant at the 0.01 probability level. ***Significant at the 0.001 probability level.

Table 1.3 Grain yield components at harvest from multiple locations and years across the U.S. for a single dicamba exposure at 2.8 g ae ha^{-1} in soybean across multiple growth stages.

Main Effects	**Nodes $Plant^{-1}$**	**Nodes with Pods**	**Pods $Plant^{-1}$**	**Seeds $Plant^{-1}$**	**Seeds Pod^{-1}**	**Grams 100 $Seed^{-1}$**
Non-treated	23	18 a[a]	52 a	119 a	2.2 ab[c]	13.93
V3	23 (0)[b]	18 (0) ab	49 (6) ab	103 (13) bc	2.1 (5) bc	14.42 (-4)
R1	21 (9)	16 (11) b	44 (15) b	89 (25) c	2.0 (9) c	14.62 (-5)
R3	21 (9)	16 (11) b	47 (10) ab	99 (17) bc	2.1 (8) c	14.21 (-2)
R5	23	18 a	52 a	119 a	2.3 ab	13.93
				ANOVA		
				F Value and Significance Level		
Single Application	2.07	2.61*	2.55*	4.79***	5.94***	1.29

[a] Within columns and by main effect, means followed by the same letter are not significantly different.
[b] Value in parentheses is percent reduction in relation to the non-treated.
[c] Different letter groupings on numbers due to rounding.
*Significant at the 0.05 probability level. **Significant at the 0.01 probability level. ***Significant at the 0.001 probability level.

Table 1.4 Grain yield, plant height at harvest, and visual plant injury at harvest in soybean from multiple locations and years across the U.S. receiving two dicamba exposures at 2.8 g ae ha^{-1} across multiple growth stages.

Main Effects	**Yield**	**Plant Height at Harvest**[a]	**Visual Injury at Harvest**[b]
	kg ha^{-1}	cm	%
Non-treated	3964 a[c]	97 a	0 e
R1	3412 (14) c[d]	77 (21) cd	19 bc
V3&R1	3215 (19) d	78 (20) cd	20 b
V3&R3	3310 (16) d	78 (20) cd	21 b
V3&R5	3674 (7) b	87 (10) b	6 d
R1&R3	2498 (37) e	63 (35) e	29 a
R1&R5	3339 (16) cd	74 (24) d	21 b
R3&R5	3612 (9) b	79 (19) c	15 c
		ANOVA	
		F Value and Significance Level	
Double Application	27.73***	37.00***	30.02***

[a] Heights recorded in centimeters from ground to apical meristem.
[b] Visual injury ratings on a scale of 0 to 100% with 0%=no injury and 100%=complete death.
[c] Within columns and by main effect, means followed by the same letter are not significantly different.
[d] Value in parentheses is percent reduction in relation to the non-treated.
*Significant at the 0.05 probability level. **Significant at the 0.01 probability level. ***Significant at the 0.001 probability level.

Table 1.5 Grain yield components at harvest from multiple locations and years across the U.S. for two dicamba exposures at 2.8 g ae ha^{-1} in soybean across multiple growth stages.

Main Effects	Nodes $Plant^{-1}$	Nodes with Pods	Pods $Plant^{-1}$	Seeds $Plant^{-1}$	Seeds Pod^{-1}	Grams 100 $Seed^{-1}$
Non-treated	23	18 ab[a]	52 a	119 a	2.2 a[c]	13.96 d
R1	21 (9)[b]	16 (11) bc	44 (15) bcd	89 (25) d	2.0 (9) cd	14.66 (-5) abc
V3&R1	22 (4)	16 (11) bc	44 (15) bcd	89 (25) d	2.0 (8) cd	13.98 (0) d
V3&R3	21 (9)	16 (11) bc	42 (19) d	85 (29) d	2.1 (8) bcd	14.15 (-1) bcd
V3&R5	23 (0)	17 (6) ab	48 (8) ab	103 (13) b	2.2 (2) ab	15.04 (-8) a
R1&R3	20 (13)	15 (17) c	44 (15) bcd	83 (30) d	1.9 (13) d	14.04 (-1) cd
R1&R5	21 (9)	16 (11) bc	43 (17) cd	90 (24) cd	2.1 (6) bc	14.38 (-3) bcd
R3&R5	21 (9)	16 (11) bc	48 (8) abc	101 (15) bc	2.1 abc (4)	14.77 (-6) ab
			ANOVA			
			F Value and Significance Level			
Double Application	1.98	2.31*	3.48**	8.95***	3.87***	3.17**

[a] Within columns and by main effect, means followed by the same letter are not significantly different.
[b] Value in parentheses is percent reduction in relation to the non-treated.
[c] Different letter groupings on numbers due to rounding.
*Significant at the 0.05 probability level. **Significant at the 0.01 probability level. ***Significant at the 0.001 probability level.

Table 1.6 Grain yield, plant height at harvest, and visual plant injury at harvest in soybean from multiple locations and years across the U.S. receiving three or more dicamba exposures at 2.8 g ae ha^{-1} across multiple growth stages.

Main Effects	Yield	Plant Height at Harvest[a]	Visual Injury at Harvest[b]
	kg ha^{-1}	cm	%
Non-treated	3945 a[c]	97 a	0 d
R1&R3	2924 (26) c[d]	63 (35) c	29 bc
V3&R1&R3	2825 (28) c	62 (36) c	38 a
V3&R1&R5	3216 (18) b	75 (23) b	24 c
V3&R3&R5	3358 (15) b	74 (24) b	25 c
R1&R3&R5	2977 (25) c	62 (36) c	31 b
V3&R1&R3&R5	2819 (29) c	61 (37) c	41 a
		ANOVA	
		F Value and Significance Level	
Three Plus Applications	31.21***	92.55***	49.17***

[a] Heights recorded in centimeters from ground to apical meristem.
[b] Visual injury ratings on a scale of 0 to 100% with 0%=no injury and 100%=complete death.
[c] Within columns and by main effect, means followed by the same letter are not significantly different.
[d] Value in parentheses is percent reduction in relation to the non-treated.
*Significant at the 0.05 probability level. **Significant at the 0.01 probability level. ***Significant at the 0.001 probability level.

Table 1.7 Grain yield components at harvest from multiple locations and years across the U.S. for three or more dicamba exposures at 2.8 g ae ha-1 in soybean across multiple growth stages.

Main Effects	**Nodes Plant^{-1}**	**Nodes with Pods**	**Pods Plant^{-1}**	**Seeds Plant^{-1}**	**Seeds Pod^{-1}**	**Grams 100 Seed^{-1}**
Non-treated	23 a[a]	18 a	52 a	119 a	2.1 a[c]	13.96
R1&R3	20 (13) bc[b]	15 (17) b	44 (15) b	83 (30) b	1.9 (13) bc	14.04 (-1)
V3&R1&R3	21 (9) bc	15 (17) b	42 (19) b	83 (30) b	1.9 (13) bc	14.41 (-3)
V3&R1&R5	21 (9) abc	16 (11) b	41 (21) b	83 (30) b	1.9 (9) b	14.34 (-3)
V3&R3&R5	21 (9) ab	16 (11) b	44 (15) b	81 (32) b	1.8 (15) bc	14.85 (-6)
R1&R3&R5	19 (17) c	15 (17) b	41 (21) b	78 (34) b	1.8 (16) c	14.84 (-6)
V3&R1&R3&R5	21 (9) abc	16 (11) b	41 (21) b	76 (36) b	1.8 (15) bc	15.22 (-9)
			ANOVA			
			F Value and Significance Level			
Three Plus Applications	2.46*	4.14***	5.49***	11.68***	6.09***	1.76

[a] Within columns and by main effect, means followed by the same letter are not significantly different.
[b] Value in parentheses is percent reduction in relation to the non-treated.
[c] Different letter groupings on numbers due to rounding.
*Significant at the 0.05 probability level. **Significant at the 0.01 probability level. ***Significant at the 0.001 probability level.

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CHAPTER II

RESPONSE OF SOYBEAN TO MULTIPLE, SUBLETHAL 2,4-D EXPOSURES FROM VEGETATIVE THROUGH REPRODUCTIVE GROWTH

Abstract

Crop yields are adversely affected by off-target movement of herbicides each year. This study was performed to ascertain whether multiple, sublethal herbicide exposures affect crop productivity. The effect of 2,4-D application timing on soybean (*Glycine max*) injury, growth, development, and yield was investigated near Fayetteville, Arkansas; De Soto, Illinois; Lafayette, Indiana; Starkville, Mississippi; Columbia, Missouri; North Platte, Nebraska; and South Charleston, Ohio on soil textures ranging from sandy loam to silty clay loam. A single application of 2,4-D at 5.6 g ae ha^{-1} applied to V3, R1, R3, or R5 soybean did not affect grain yield. Conversely, multiple 2,4-D exposures at the V3&R1, V3&R3, or R1&R3 growth stages reduced yield up to 7%. Three or more applications of 2,4-D had no further effect on yield relative to exposing soybean to 2,4-D twice between V3 and R3. Sublethal applications of 2,4-D can reduce soybean grain yield if two or more occur between V3 and R3.

Introduction

As weeds develop resistance to current labeled herbicides, companies are turning to some of agriculture's oldest herbicides for results. Soybean tolerant to 2,4-D will add another method of control for increasingly difficult to control weeds and is expected to be widely adopted throughout the United States (Mortensen et al. 2012). Recently, these systems have provided good broadleaf weed control, but extensive off-target movement (OTM) and sensitive vegetation injury has been reported (Bradley 2017; Mueller and Steckel 2021).

While little research exists related to OTM of 2,4-D onto soybean, multiple studies have evaluated the effect of dicamba on soybean. This is largely due to the fact that soybean is far more sensitive to dicamba than to 2,4-D; however, sensitivity often depends on growth stage at the time of exposure (Wax et al. 1969; Andersen et al. 2004; Sciumbato et al. 2004; Kelley et al. 2005; Johnson et al. 2012; Robinson et al. 2013; Egan et al. 2014; Scholtes et al 2019). Over the past couple of years, sensitive vegetation, specifically soybean, has been subjected to OTM of herbicides (Bradley 2017; Dowell 2017; Hager 2017; Hartzler 2017; Zaccaro et al. 2020). Non-auxin tolerant soybean grown in proximity to soybean receiving an auxin herbicide application may be exposed to OTM multiple times during the growing season. The flowering stages of soybean have been found by previous research to be most sensitive with respect to yield loss when compared to vegetative or later reproductive stages (Wax et al. 1969; Auch and Arnold 1978; Griffin et al. 2013; Robinson et al. 2013; Solomon and Bradley 2014; Jones et al. 2019a). Kelley et al. (2005) reported lower soybean yields after 2,4-D exposure at V3 than at V7.

The severity of soybean injury following exposure to an auxin herbicide can range from mild to severe and is influenced by many factors including dose, growth stage at time of exposure, and inherent susceptibility to the auxin herbicide. As with dicamba, exposure to a sublethal dose of 2,4-D causes soybean injury symptoms such as epinasty, leaf strapping, and stem callousing (Wax et al 1969; Al-Khatib and Peterson 1999; Andersen et al. 2004; Sciumbato et al. 2004; Egan et al. 2014; Jones et al. 2019b; Werle et al, 2021). Pod malformation also has been observed when low doses of auxin are applied to soybean during reproductive stages (McCown et al. 2018). Minimal literature exists examining the effects of a single exposure of 2,4-D on soybean. This, coupled with the absence of literature investigating the effects of multiple, sublethal 2,4-D exposures, resulted in the objective of this study: to ascertain whether multiple, sublethal 2,4-D exposures affect soybean productivity in comparison to a single, sublethal exposure.

Materials and Methods

Experiments were conducted from 2019 to 2021 at seven locations across the United States to assess the susceptibility of non-2,4-D tolerant soybean to 2,4-D exposure. Soil textures, row spacing, planting date, and soybean cultivar varied by location and are delineated in Table 2.1. At each location, the experimental design was a randomized complete block with four replications and plots four rows wide. Treatments consisted of 2,4-D (Enlist One™, Corteva™) applications at 5.6 g ae ha^{-1} to non-2,4-D tolerant soybean at every possible combination of the growth stages V3, R1, R3, and R5. Applications at V3 were made to soybean with three to four fully expanded trifoliates. Treatments applied to reproductive growth stage soybean were made at R1, (initial flowering or a flower located anywhere on the plant); R3, (initial pod set or a 3/16" long pod in the upper four nodes); and R5, (pod fill or visible seed in one of the pods of the upper four nodes). A non-exposure treatment was included at each growth stage resulting in several treatments receiving only a single V3, R1, R3, or R5 application. Applications were made using a CO_2 pressurized backpack sprayer with TTI nozzles (Teejet Spraying Systems, Wheaton IL) calibrated to deliver 140 L ha^{-1}. Data collection included visual evaluation of soybean injury, heights, yield, and yield components including nodes $plant^{-1}$, nodes with a pod, pods $plant^{-1}$, seeds $plant^{-1}$, and grams 100 $seed^{-1}$. Visual injury and height data were collected at fourteen and twenty-eight days after each treatment as well as at soybean maturity. At maturity, yield components from ten plants in each plot were collected for evaluation prior to machine harvest. Yield was collected from the center two rows of each plot with moisture corrected to 13% for yield calculations.

Analysis of variance was conducted using the GLIMMIX procedure in SAS 9.4 (SAS Institute Inc., Cary, NC). Data were analyzed as a mixed model with 2,4-D application as a fixed effect and replication, location, and year as random effects. Three predetermined comparisons were performed to meet the objectives of this experiment. First, all treatments with a single application of 2,4-D were compared to determine the growth stage(s) at which a single exposure of non-tolerant soybean to 2,4-D was most detrimental. Second, all double applications of 2,4-D were compared to the most detrimental single exposure of non-tolerant soybean to 2,4-D. Third, three or more 2,4-D applications to non-tolerant soybean were compared to the most yield-limiting single or double exposure. All three comparisons included a non-treated as the positive control, while investigations of multiple exposures also included the most injurious exposure at a lesser number of 2,4-D applications as the negative control. Degrees of freedom were calculated using the Kenward-Rogers method and least-square means were considered different at $\alpha \leq 0.05$ (Kenward and Rogers, 1997).

Results and Discussion

The central hypothesis of this study was that multiple, sublethal exposures of 2,4-D are more deleterious to soybean growth, development, and yield than a single exposure. A single, sublethal application of 2,4-D applied to soybean from V3 through R5 had no effect on soybean grain yield in relation to the non-treated (Table 2.2). Exposure at the V3&R1, V3&R3, or R1&R3 growth stages reduced yield 5 to 7% (Table 2.4). Three or more 2,4-D exposures on non-2,4-D tolerant soybean had no adverse effect on yield relative to exposing soybean twice from V3 through R3 (Table 2.6).

Single Exposure

Regardless of growth stage, a single application of 2,4-D did not affect soybean yield or yield components but reduced plant height and increased visual injury when applied at the R1 growth stage. A single 5.6 g ae ha^{-1} application of 2,4-D from V3 through R5 had no effect on soybean yield or yield components (Tables 2.2 and 2.3). Exposing R1 soybean to 2,4-D had no effect on mature plant height but did increase visual injury slightly. The effects of a sublethal rate of 2,4-D on plant growth, development, and yield observed in these studies are generally in agreement with existing literature.

Soybean exposure to 2,4-D at concentrations that approximate a tank contamination or an off-target movement rate has minimal effect on soybean yield. Generally, no effect on soybean yield is observed until exposures occur at a rate in excess of 56 g ae ha^{-1} (Wax et al. 1969; Andersen et al. 2004; Johnson et al. 2012; Egan et al. 2014). An application of 2,4-D ranging from 180 to 2240 g ha^{-1} to V2, V5, or R2 soybean reduced reproductive nodes m^{-2}, pods reproductive $node^{-1}$, pods m^{-2}, seeds pod^{-1}, seeds m^{-2}, and seed mass 10% (Robinson et al. 2013). In comparison to the non-treated, 2,4-D rates ranging from 0.028 to 267 g ae ha^{-1} applied to R2 soybean resulted in up to 25% visual injury and reduced plant height by as much as 21% (Wax et al. 1969; Kelley et al. 2005; Robinson et al. 2013; Solomon and Bradley 2014; Scholtes et al. 2019). Exposing soybean to a sublethal rate of 2,4-D from vegetative through early reproductive growth did not have an adverse effect on height yield or yield components but may result in injury. Soybean exposed to 2,4-D from tank contaminations or off-target movement may result in visual injury and height reduction but effect on yield is negligible.

Multiple Exposures

Exposing soybean to multiple, sublethal applications of 2,4-D may reduce yield relative to a single application. Two or more applications of 2,4-D at 5.6 g ae ha^{-1} occurring from V3 through R3 reduced soybean yield 5 to 7% (Tables 2.4 and 2.6). No adverse effects were detected in yield components with two or more applications of 2,4-D (Table 2.5 and 2.7). Multiple 2,4-D applications at 5.6 g ae ha^{-1} from vegetative through early reproductive stages may cause moderate phenoxy symptomology, alterations in yield components, and moderate reductions in soybean yield.

Conclusion

This research was conducted to determine whether non-2,4-D tolerant soybean productivity is adversely affected by multiple, sublethal 2,4-D exposures. Regardless of growth stage, a single 5.6 g ae ha^{-1} application rate had no effect on soybean grain yield. Conversely, multiple 2,4-D applications at 5.6 g ae ha^{-1} from vegetative through early reproductive stages may cause slight phenoxy symptomology, alterations in yield components, and a moderate yield loss. Non- 2,4-D tolerant soybean exposed to 2,4-D from tank contaminations or off-target movement may result in visual injury and height reductions and effects on yield will be negligible.

Table 2.1 State, year, latitude, longitude, soil texture, maturity group, variety, planting date, population, and row spacing of all locations for a soybean study examining the comparison between one, two, and three plus exposures of 5.6 g ae ha^{-1} 2,4-D at multiple growth stages.

State	Year	Latitude	Longitude	Soil	MG[a]	Variety	Planting Date	Seed Hectare^{-1}	Row Spacing (cm)
Arkansas	2020	36.0994	-94.179	silt loam	4.4	Credenz 4410 GTLL	June-12-2019	345947	91
Illinois	2019	37.7954	-89.2601	silt loam	3.7	Pioneer P37T09L	May-25-2019	345947	76
	2020	37.7069	-89.2525	silt loam	3.9	Asgrow 39X7	May-24-2020	345947	76
Indiana	2020	40.2972	-86.9035	silt loam	3.4	DynaGrow S34GL79	May-24-2020	345947	76
Missouri	2019	38.8915	-92.2078	silt loam	3.6	Golden Harvest 3625L	June-5-2019	345947	76
	2020	38.8915	-92.2078	silt loam	-	-	-	345947	76
Mississippi	2019	33.4743	-88.7716	sandy loam	4.6	Asgrow 46X6	May-2-2018	345947	76
	2020	33.4743	-88.7716	sandy loam	4.6	Asgrow 46X6	May-19-2020	345947	76
	2021	33.4743	-88.7716	sandy loam	4.7	Asgrow 47XF0	May-17-2021	345947	76
Nebraska	2019	41.0899	-100.7675	silt loam	2.6	Asgrow 2636	May-17-2019	345947	76
Ohio	2019	39.8587	-83.6706	silt loam	3.3	Seed Consultants 3357LL	June-4-2019	358302	76
	2020	39.86093	-83.6728	silt loam	3.3	Seed Consultants 3319LL	May-13-2020	395368	76

[a] MG is an abbreviation for Maturity Group

Table 2.2 Grain yield, plant height at harvest, and visual plant injury at harvest in soybean from multiple locations and years across the U.S. receiving one 2,4-D exposure at 5.6 g ae ha^{-1} across multiple growth stages.

Main Effects	**Yield**	**Plant Height at Harvest**[a]	**Visual Injury at Harvest**[b]
	kg ha^{-1}	cm	%
Non-treated	3827[c]	85	0 b
V3	3771 (1)[d]	84 (1)	1 b
R1	3763 (2)	80 (6)	2 a
R3	3784 (1)	85 (0)	0 b
R5	3746 (2)	83 (2)	0 b
		ANOVA	
		F Value and Significance Level	
Single Application	<1	2.31	4.30**

[a] Heights recorded in centimeters from ground to apical meristem.
[b] Visual injury ratings on a scale of 0 to 100% with 0%=no injury and 100%=complete death
[c] Within columns and by main effect, means followed by the same letter are not significantly different.
[d] Values in parentheses is percent reduction in relation to the non-treated
*Significant at the 0.05 probability level. **Significant at the 0.01 probability level. ***Significant at the 0.001 probability level.

Table 2.3 Grain yield components at harvest from multiple locations and years across the U.S. for a single 2,4-D exposure at 5.6 g ae ha^{-1} in soybean across multiple growth stages.

Main Effects	Nodes $Plant^{-1}$	Nodes with Pods	Pods $Plant^{-1}$	Seeds $Plant^{-1}$	Seeds Pod^{-1}	Grams 100 $Seed^{-1}$
Non-treated	24[a]	21	45	94	2.0[c]	15.22
V3	26 (-8)[b]	21 (0)	48 (-7)	102 (-9)	2.0 (-1)	14.91 (2)
R1	24 (0)	19 (10)	45 (0)	94 (0)	2.0 (0)	14.9 (2)
R3	26 (-8)	21 (0)	48 (-7)	100 (-6)	2.0 (1)	15.15 (0)
R5	25 (-4)	21 (0)	47 (-4)	101 (-7)	2.1 (-3)	15.41 (-1)
	ANOVA					
	F Value and Significance Level					
Single Application	1.85	2	<1	1.05	<1	<1

[a] Within columns and by main effect, means followed by the same letter are not significantly different.
[b] Values in parentheses is percent reduction in relation to the non-treated.
[c] Different letter groupings on numbers due to rounding.
*Significant at the 0.05 probability level. **Significant at the 0.01 probability level. ***Significant at the 0.001 probability level.

Table 2.4 Grain yield, plant height at harvest, and visual plant injury at harvest in soybean from multiple locations and years across the U.S. receiving two 2,4-D exposures at 5.6 g ae ha^{-1} across multiple growth stages.

Main Effects	Yield	Plant Height at Harvest[a]	Visual Injury at Harvest[b]
	kg ha^{-1}	cm	%
Non-treated	3830 a[c]	85	0 b
V3&R1	3590 (6) c[d]	81 (5)	2 ab
V3&R3	3642 (5) bc	85 (0)	1 b
V3&R5	3842 (0) a	83 (2)	1 b
R1&R3	3549 (7) c	79 (7)	2 a
R1&R5	3863 (-1) a	83 (2)	2 ab
R3&R5	3816 (0) ab	83 (2)	0 b
		ANOVA	
		F Value and Significance Level	
Double Application	4.40***	1.41	2.39*

[a] Heights recorded in centimeters from ground to apical meristem.
[b] Visual injury ratings on a scale of 0 to 100% with 0%=no injury and 100%=complete death
[c] Within columns, means followed by the same letter are not significantly different.
[d] Values in parentheses is percent reduction in relation to the non-treated
*Significant at the 0.05 probability level. **Significant at the 0.01 probability level. ***Significant at the 0.001 probability level.

Table 2.5 Grain yield components at harvest from multiple locations and years across the U.S. for two 2,4-D exposures at 5.6 g ae ha^{-1} in soybean across multiple growth stages.

Main Effects	Nodes $Plant^{-1}$	Nodes with Pods	Pods $Plant^{-1}$	Seeds $Plant^{-1}$	Seeds Pod^{-1}	Grams 100 $Seed^{-1}$
Non-treated	24	21	45 ab[a]	94	2.0[c]	15.22
V3&R1	25 (-4)[b]	20 (5)	45 (0) ab	98 (-4)	2.1 (-4)	14.7 (3)
V3&R3	25 (-4)	20 (5)	45 (0) ab	96 (-2)	2.1 (-4)	14.95 (2)
V3&R5	25 (-4)	21 (0)	46 (-2) a	95 (-1)	2.0 (2)	16.85 (-11)
R1&R3	23 (4)	20 (5)	41 (9) b	89 (5)	2.0 (-3)	15.15 (0)
R1&R5	25 (-4)	21 (0)	47 (-4) a	100 (-6)	2.0 (-2)	14.67 (4)
R3&R5	26 (-8)	21 (0)	49 (-9) a	100 (-6)	1.9 (3)	15.18 (0)
			ANOVA			
			F Value and Significance Level			
Double Application	1.47	<1	2.21*	1.3	<1	<1

[a] Within columns and by main effect, means followed by the same letter are not significantly different.
[b] Values in parentheses is percent reduction in relation to the non-treated
[c] Different letter groupings on numbers due to rounding.
*Significant at the 0.05 probability level. **Significant at the 0.01 probability level. ***Significant at the 0.001 probability level.

Table 2.6 Grain yield, plant height at harvest, and visual plant injury at harvest in soybean from multiple locations and years across the U.S. receiving three or more 2,4-D exposures at 5.6 g ae ha^{-1} across multiple growth stages.

Main Effects	Yield	Plant Height at Harvest[a]	Visual Injury at Harvest[b]
	kg ha^{-1}	cm	%
Non-treated	3830 a[c]	85 ab	0 c
V3&R1	3590 (6) c[d]	81 (5) abc	2 abc
V3&R3	3642 (5) bc	85 (0) ab	1 bc
R1&R3	3549 (7) c	79 (7) c	2 a
V3&R1&R3	3555 (7) c	78 (8) c	3 a
V3&R1&R5	3659 (4) abc	81 (5) abc	2 ab
V3&R3&R5	3803 (1) ab	82 (4) abc	1 bc
R1&R3&R5	3685 (4) abc	80 (6) bc	3 a
V3&R1&R3&R5	3666 (4) abc	81 (5) abc	3 a
		ANOVA	
		F Value and Significance Level	
Three Plus Applications	2.31*	2.00*	3.59***

[a] Heights recorded in centimeters from ground to apical meristem.
[b] Visual injury ratings on a scale of 0 to 100% with 0%=no injury and 100%=complete death
[c] Within columns and by main effect, means followed by the same letter are not significantly different.
[d] Values in parentheses is percent reduction in relation to the non-treated.
*Significant at the 0.05 probability level. **Significant at the 0.01 probability level. ***Significant at the 0.001 probability level.

Table 2.7 Grain yield components at harvest from multiple locations and years across the U.S. for three or more 2,4-D exposures at 5.6 g ae ha^{-1} in soybean across multiple growth stages.

Main Effects	Nodes $Plant^{-1}$	Nodes with Pods	Pods $Plant^{-1}$	Seeds $Plant^{-1}$	Seeds Pod^{-1}	Grams 100 $Seed^{-1}$
Non-treated	24[a]	21	45	94	2.0[c]	15.22
V3&R1	25 (-4)[b]	20 (5)	45 (0)	98 (-4)	2.1 (-3)	14.7 (3)
V3&R3	25 (-4)	20 (5)	45 (0)	96 (-2)	2.1 (-3)	14.95 (2)
R1&R3	23 (4)	20 (5)	41 (9)	89 (5)	2.1 (-3)	15.15 (0)
V3&R1&R3	25 (-4)	20 (5)	47 (-4)	98 (-4)	2.0 (2)	14.73 (3)
V3&R1&R5	26 (-8)	21 (0)	47 (-4)	102 (-9)	2.1 (-2)	14.81 (3)
V3&R3&R5	25 (-4)	20 (5)	45 (0)	95 (-1)	2.1 (-1)	15.17 (0)
R1&R3&R5	23 (4)	19 (10)	44 (2)	89 (5)	2.0 (2)	14.85 (2)
V3&R1&R3&R5	25 (-4)	21 (0)	46 (-2)	98 (-4)	2.0 (0)	15.23 (0)
	ANOVA					
	F Value and Significance Level					
Three Plus Applications	1.85	<1	1.22	1.13	<1	<1

[a] Within columns and by main effect, means followed by the same letter are not significantly different.
[b] Values in parentheses is percent reduction in relation to the non-treated
[c] Different letter groupings on numbers due to rounding.
*Significant at the 0.05 probability level. **Significant at the 0.01 probability level. ***Significant at the 0.001 probability level.

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