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Determining the Effect of Auxin Herbicide Concentration and Application Timing on Soybean (Glycine Max) Growth and Yield

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Determining the effect of auxin herbicide concentration and application timing on soybean (Glycine max) growth and yield

By

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Auxin resistant cropping systems will provide producers with an alternative option for weed management, but with this new technology also comes the concern of off target movement of dicamba and/or 2,4-D to susceptible crops. Research was conducted over multiple site years in order to determine the effect of soybean response to different application timings and rates of 2,4-D and dicamba. 2,4-D was applied at 1X (0.56 kg ae/ha), 1/4X, 1/16X, 1/64X, and 1/256X rates, and dicamba was applied in a separate study at 1X (0.56 kg ae/ha), 1/4X, 1/16X, 1/64X, 1/256 and 1/1024X. All rates were applied at the V3 and R1 growth stages. Greatest yield losses occurred from dicamba applied at the R1 growth stage. Additional studies were conducted to determine at which growth stage soybeans are most sensitive to 2,4-D and dicamba. Greatest yield losses occurred at the late vegetative and early reproductive growth stages for both herbicides.

DEDICATION

The dedication of this research first and foremost goes to my Savior, Jesus Christ; to God be the glory. Next I would like to dedicate this research to my mother, Gwen Blaine. Her constant love, support, and encouragement has molded me into the woman I am today, and for that I am forever grateful. I would also like to dedicate this to my father, Dr. Alan Blaine. His guidance throughout my graduate school career has been of great value and I am very thankful for all the support and knowledge that he has instilled in me. Lastly, I would like to dedicate this research to my husband, Beck Scholtes. His constant support, encouragement, and selfless sacrifices have made this all possible. Thank you so much for always being patient with me. I love you all very much.

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CHAPTER I

INTRODUCTION

Soybeans [*Glycine max* (*L.*) Merr.] are considered to be a very important crop for the state of Mississippi; it is a leading commodity for this state due to its value in the economy (USDA-MDAC 2012). Soybean production provides a significant contribution to the agricultural economy in the southeastern United States (Heatherly and Hodges 1999). In 2013, the state of Mississippi harvested over 1.9 million acres of soybeans with an average of 45 bushels per acre at an average monetary value of $14.00 per bushel (USDA-NASS 2014).

Soybeans are annual dicots that are considered to be sparsely branched, with a bush-like growth habit (Hymowitz and Singh 1987). The development of a soybean is a continuous process that begins at seed germination and is completed when seeds are mature and ready for harvest (Fehr and Caviness 1977). Separate descriptions are used to identify stages of vegetative growth and reproductive growth. Vegetative stages are described from the time the plant emerges from the soil to when the plant begins to produce flowers; once flowering begins the growth stages are now considered to be reproductive growth (Fehr and Caviness 1977). By standards of the Fehr and Caviness (1977) soybean developmental scale, a soybean plant is to be considered physiologically mature and ready for harvest when over fifty percent of the pods on the plant are mature in color.

Weed control is very important when trying to produce a high yielding crop of soybeans. Weed control in soybeans prior to the herbicide tolerant seed traits required a great amount of effort. In order to produce a high yielding and high quality soybean crop, a combination of biological, cultural, mechanical, and chemical weed control methods were necessary. With herbicide resistant cropping systems, a high yielding and high quality crop is a goal that is easier to achieve by using just chemical control alone.

In 1996, glyphosate tolerant cropping systems were adopted by producers in order to achieve greater efficiency in their production systems. By 2008, over 90% of the United States' soybean crop was planted in herbicide resistant seed varieties (USDA-NASS 2008). With this quick adoption of glyphosate-resistant cropping systems came an overreliance on glyphosate for weed control. This increased glyphosate usage brought with it an increase in glyphosate-resistant weeds (Nandula 2010). Herbicide resistant weeds make it more difficult to control weed populations in cropping systems. Herbicide resistance is believed to have evolved by two mechanisms; one being target site resistance, which is where high application rates of a single herbicide have been applied (Zelaya and Owen 2004). The other way in which resistant weeds are believed to have evolved is from reduced application rates of a given herbicide over a period of time (Gressel 1995). As of 2013, 28 weed species have been reported to be glyphosate-resistant around the world (Heap 2014). Due to this increasing number of glyphosate resistant weeds, producers are now relying on older modes of action to control weeds in their cropping systems.

In 2005, Monsanto Company announced that they were in the developmental stage of producing dicamba-tolerant seed traits under a license agreement with the

Department of Biochemistry at the University of Nebraska, Lincoln (Behrens et al. 2007). According to a press release by the company, this technology (Round Up® 2 Xtend) is currently scheduled for commercial release pending regulatory approval (Monsanto Company 2014). Additionally, Dow AgroSciences announced that they had begun development of the Enlist™ Weed Control System. This particular cropping system is a 2,4-D resistant cropping system; this seed technology is also currently scheduled for commercial release pending regulatory approval (Randolph and Barr 2014). These new seed technologies will allow for auxin herbicides to be foliar applied to soybeans. With this additional mode of action available in a soybean cropping system, producers may be able to better control glyphosate resistant weeds. Applications of auxins have shown great activity for Palmer amaranth [*Amaranthus palmeri* (S.) Wats.] (Zelaya and Owen 2004). Palmer amaranth is a weed that has been particular problematic in mid-south soybean production systems because of its evolved resistance to glyphosate and other herbicides (Nandula 2010).

Auxinic herbicides, such as 2,4-D and dicamba, are relatively inexpensive to use and have little soil residual activity (Senseman 2007). These herbicides have been extensively used for weed control for over 60 years primarily due to their selectivity, wide spectrum of weed control, efficacy, and low application costs (Mithila et al. 2011). Auxin herbicides mimic natural occurring auxin, which is a plant growth hormone that is central to the control of plant growth and development (Abel and Theologis 1996). Auxin herbicides, also commonly known as synthetic auxins, mimic the plant growth hormone indole-3-acetic acid (IAA); mimicking IAA disrupts growth and development processes, eventually causing plant death (Senseman 2007). Auxin herbicides are readily taken up by the roots and foliage and are translocated in the both the phloem and xylem of the plants to which they are applied to. According to *The Herbicide Handbook* (Senseman 2007), 2,4-D controls broadleaf species such as carpetweed (*Mollugo verticillata*), horseweed (*Conzya canadensis*), pigweed (*Amaranthus spp.*), and velvetleaf (*Abutilon theophrasti*), among many other problematic weed species that can be found in a cropping system. Dicamba is most commonly used to control annual broadleaf weeds such as pigweed *(Amaranthus spp*.), wild buckwheat (*Polygonum convolvulus*), and lambsquarters (*Chenopodium album*); higher rates of dicamba are capable of controlling perennial broadleaf weeds such as field bindweed (*Convolvulus arvensis*) (Senseman 2007). Symptomology that can be observed from auxin herbicides include: swelling of the stems, cupping of the leaves, epinastic twisting of the stems and petioles of plants, chlorosis, and/or necrosis (Senseman 2007; Wax et al. 1969; Robinson(a) et al. 2013; Robinson(b) et al. 2013; Egan et al. 2014).

Dicamba and 2,4-D have been widely used for many decades and little development of auxin resistant weeds have been recorded (Nandula 2010). With only a few weed species that have developed resistance to auxin herbicides, producers are going full circle and are once again relying on these older modes of action to control problematic weeds in their cropping systems. The introduction of dicamba and 2,4-D tolerant crops may offer producers a way to control glyphosate-resistant weed species and other weeds that may be difficult to control. While offering many potential advantages, these herbicide tolerant traits will also bring many new challenges. These herbicides have the potential to greatly damage any susceptible crops and possibly result in a severe yield loss (Egan et al. 2014). Dicamba and 2,4-D applications have the potential to not only physically drift to susceptible plant species (Egan et al. 2014), but they also have the potential to volatilize to off target application areas (Strachan et al. 2013). 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 due to improper application practices (Johnson, W. et al. 2012). Producers who choose to utilize these technologies will have to use great care to prevent damage susceptible crops.

Previous research in cotton has indicated that a yield loss can occur due to exposure of 2,4-D or dicamba (Smith et al. 2010). Smith et al. (2010) found that significant yield reductions were observed from both 2,4-D and dicamba; however, 2,4-D was more injurious to cotton when compared to dicamba. The results of this experiment and similar experiments (Smith et al. 2010; Marple et al. 2008; Everitt et al. 2009) suggest that cotton is more sensitive to 2,4-D than dicamba, whereas other studies (Andersen et al. 2004; Johnson, V. et al. 2012) suggest that soybeans are more sensitive to dicamba versus 2,4-D. Cotton yield losses were observed where minimal visual injury from exposure to 2,4-D was present (Smith et al. 2010). Due to the results of these experiments, we can assume that it is likely that soybeans would also experience similar yield losses.

With increasing in-season, broadcast applications of 2,4-D and dicamba in the near future, many have become concerned with the effects that may occur to crops that are susceptible to auxins. With this in mind, chemical companies are considering herbicide application requirements (Randolph and Barr 2014). These requirements will allow producers to make applications only with spray tips that put out a very course to ultra-course droplet size, only making applications in wind speeds less than ten miles per hour, advising to make applications only when conditions are favorable to avoid temperature inversions, and also to require a buffer between areas of application and susceptible crop species (Johnson, V. et al. 2012). With these requirements only being proposed by the companies as a means to avoid any potential damage to off target species; we must look at what could happen if off target movement occurs, if not by particle drift or volatilization then by means of sprayer contamination.

The objectives of this research were to assess under field conditions (1) the effect of low concentrations of both dicamba and 2,4-D on soybean growth and yield, and also (2) to determine at which growth stage soybeans are most sensitive to exposure to dicamba and 2,4-D. Separate experiments have been conducted for each individual herbicide, dicamba and 2,4-D.

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CHAPTER II

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

Abstract

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. Trace amounts of 2,4-D on sensitive species can result in severe injury or even death to the plant. 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 other crops.

The dimethylamine (DMA) salt of 2,4-D was used to evaluate the effect of application timing and rate of application 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 both the V3 and R1 soybean growth stages. In another experiment an application of 1/4X rate (0.14 kg ae/ha) of 2,4-D was applied on weekly intervals until the soybeans reached physiological maturity. Soybean growth stage was recorded at each application in order to determine the most sensitive application timing.

Visual injury estimates, plant heights, and yield data were collected for all experiments. Soybean yield reductions were significant at both application rate and

timing, the interaction of rate by timing was also found to be significant. Applications of 2,4-D applied at 1X , 1/4X, 1/16X, 1/64X, and 1/256X rates resulted in 65, 20, 9, 11, and 2% yield reduction, respectively, when applied at the V3 growth stage. 2,4-D applied at 1X, 1/4X, 1/16X, 1/64X, and 1/256X rates resulted in a 32, 12, 6, 5, and 8% yield reduction, respectively, when applied at the R1 growth stage.

The greatest visual injury from 0.14 kg ae/ha of 2,4-D in the application timing experiment (33%) observed at 14 DAT was at the R1 growth stage. No significant visual injury was observed for applications made after R4 growth stage, and no significant height reductions were recorded for applications made after the R4 growth stage, which corresponded with the 8 weeks after emergence application timing. Yield reductions were variable; however, the greatest yield reductions were observed when 0.14 kg ae/ha of 2,4-D was applied at the V3 through R3 growth stages; with yield reductions ranging from 17 to 27%.

Nomenclature

2,4-D Amine, 2,4-Dichlorophenoxyacetic acid, dimethylamine salt; soybeans, *Glycine max* (L.) Merr.

Abbreviations

MOA, mode of action; g ae/ha, grams acid equivalent per hectare; L/ha, liters per hectare; m, meter; kg ae/ha, kilograms of acid equivalent per hectare; DAT, days after treatment; cm, centimeter; bu/A, bushels per acre; WAE, weeks after emergence

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 is typical of most auxin herbicides; epinastic twisting of the stems and petioles, cupping and strapping of the leaves, and swelling of the stems are often observed on broadleaf plants. All of these symptoms are followed by chlorosis at the plants growing points, 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 of 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 application 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 had been exposed to 2,4-D had indicated that soybean response resulted in immediate twisting of the stems and petioles (epinastic response), slight cupping and strapping of the leaves become noticeable overtime (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 has 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 result in 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 over four locations in the southeastern United States. Experiments were conducted in order 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 when on 38 inch 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 both objective experiments discussed in this chapter.

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.

Experimental tests were conducted in the following 6 locations: 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 in AR (2013). Planting date, planting populations, 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 the application timing. Treatments were applied at 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 the rate of 2,4-D applied. Rates were based off a 1X rate of 2,4-D that was 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 titration 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 (1.9m 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 for 2013 applications. Plots were maintained as 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 7, 14, 21, and 28 DAT. Visual evaluations were collected as a percentage, ranging from 0 (0=no injury) to 100 (100=plant death), of overall soybean injury. Visual evaluations were collected at all locations with the exception of the Rohwer location. Plant heights were collected at the end of the growing season at all locations with the exception of the Rohwer location. Six plants were measured in each plot to obtain plant height data. Yield data were also 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).

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

Experiments were conducted during the growing season of 2013 to evaluate the effect of 2,4-D application timing on soybean growth and yield. Experimental tests were conducted in the following 4 locations: BlackBelt Experiment Station in Brooksville, MS (2013), R. R. Foil Plant Science Research Center in Starkville, MS (2013), Delta Research and Extension Center, Stoneville in MS (2013), and Rohwer Research Station in Rohwer, AR (2013). Planting date, planting populations, 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 (0.14 kg ae/ha) was applied at weekly intervals; this rate was equivalent to the 1/4 X rate from the previous experiment. Applications were made beginning one week after plant emergence; each additional application was made at weekly intervals until the plants began to naturally senesce. The growth stage of the soybeans were 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 as 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 7, 14, 21, and 28 DAT. Visual evaluations were collected as a percentage, ranging from 0 (0=no injury) to 100 (100=plant death), of overall soybean injury. Visual evaluations were collected at all locations with the exception of the Rohwer location. Plant heights were collected at the end of the growing season at all locations with the exception of the Rohwer location. Six plants in each 3.9 m by 12.2 m plot were measured in order to collect plant height data. Yield data were also collected from the treated area of each experimental unit 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

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

Visual injury and plant height exhibited a significant rate by application timing interaction for all ratings. Therefore, data are expressed as a function of rate and timing pooled over all locations. Overall, 2,4-D applied to soybeans greatly affected yield.

The effect of 2,4-D application timing and rate from 7, 14, 21, and 28 DAT visual injury ratings is displayed in Table 2.2. Injury ratings 7 DAT showed a 1X application of 2,4-D resulted in 56 and 45% visual injury ratings at the V3 and R1 growth stage, respectively. Significant injury was observed at all application rates with the exception of the 1/64X application rate made at the R1 application timing. Both application timings at the 1/256X rate showed no significant visual injury 7 DAT. Injury ratings 14 DAT were significant at the 1X, 1/4X, 1/16X rates at both application timings; injury ratings were also significant at the R1 application timing of the 1/64X and 1/256X rates. Significant injury ranged from 62 to 12% depending on application timing and rate. Data from the 21 DAT injury ratings were significant at all application timings and rates with the exception of the V3 application timing of the 1/256X application rate. Injury 21 DAT ranged from 63 to 12% depending on application timing and rate. Injury recorded for 28 DAT was significant for all application timings and rates with the exception of the 1/256X rate at the R1 growth stage application timing. Injury ranged from 58 to 9% depending on application timing and rate.

The interaction of timing by rate was found to be significant for plant height reductions. Plant heights were recorded in the field in centimeters (cm) and are displayed in Table 2.3. Height reductions were calculated as a percentage based on the untreated check plots using the following formula: (average check plot height – average plot height) / average check plot height (100) = percent reduction. Height reductions were greatest where higher application rates of 2,4-D were applied. Height reductions were significantly different at all application rates and timings with the exception of the 1/16X, 1/64X, and 1/256X rates at the V3 application timing. Height reductions ranged from 39 to 7% depending on application rate and timing.

Yield was found to have a significant interaction of timing by rate. Yield and percent yield reduction, which was calculated as a percentage from the yield collected of the untreated check plots; this can be viewed in Table 2.4. Yield (kg/ha) was significant at the 1X and 1/4X application rates of 2,4-D at both application timings (V3 and R1). Yield ranged from 1263 to 3289 kg/ha depending on the application timing and rate. Yield reductions were also significant for the interaction of timing by rate. Significant yield reductions were observed at both application timings for the 1X and the 1/4X rate, for the V3 application timing at the 1/16X and 1/64X rate, and also at the 1/256X rate from the R1 application timing. Yield reductions from the 1X application rate of 2,4-D were determined to be 65 and 32% at the V3 and R1 growth stages, respectively. Yield reductions from the 1/4X application rate were 20 and 12% at the V3 and R1 growth stages, respectively. Yield reductions were also significant at the 1/16X and 1/64X rates when applied at the V3 growth stage, 9 and 11%, respectively. The 1/256X rate of 2,4-D applied at the R1 growth stage also resulted in a significant yield loss of 8%.

These data indicate that higher rates of 2,4-D will result in greater visual injury, height reductions, and yield reductions. Soybeans that have been exposed to the labeled rate (1X) of 2,4-D can experience up to a 65% yield loss, depending on when that exposure to the herbicide occurred. These data also indicate that where greater visual injury and height reductions were present we could also expect to see a greater yield reduction. Based on the visual injury data collected, it was observed that soybean injury was reduced overtime; 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 at lower application rates has very little potential to cause 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 we initially thought they would be. Similarly to the findings of Robinson et al (2013), this study indicates that soybean yield reductions were only effected by higher application rates.

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

Growth stage of the soybeans at each weekly application timing was significant at all visual injury rating dates, plant heights (cm), height reductions, yield (kg/ha), and yield reductions. All visual injury data can be viewed in Table 2.5, and all plant height and yield data can be viewed in Table 2.6. Visual injury at 7 DAT was significant at applications made at the VE through R4 and at the R5.5 growth stage. Visual injury from 7 DAT was greatest at the V4, V5, R1, R2, and R3 growth stages with visual injury ratings of 25,25,34,32, and 31%, respectively. Visual injury ratings 14 DAT were found to be significant at applications made at the VE through V4 growth stages. Greatest injury recorded from 14 DAT was 25, 33, 31, and 26%, which corresponds with the V4, R1, R2, and R3 growth stages, respectively. Injury ratings observed 21 DAT were significant at the VE through R4 growth stages. The greatest amount of injury that was observed from 21 DAT visual injury ratings were at the V2, V4, V5, R1, R2, R3, and R4 growth stages, which were at 17, 27, 19, 27, 24, 22, and 25%, respectively. Injury ratings from the final rating date at 28 DAT were significant at applications made at the VE through V4 growth stages. Visual injury was greatest at 28 DAT from the applications of 2,4-D made at the V2, V3, V4, V5, R1, R2, R3, and R4 growth stages, which were recorded to be 16, 20, 23, 24, 26, 20, 18, and 18% injury, respectively. Overall, no significant visual injury was observed after the R5 growth stage at 21 DAT and 28 DAT. The R5 growth stage is the growth stage at which pod fill begins to occur (Fehr and Caviness 1977).

Plant heights were significantly less than the untreated check at the VE, V2, V3, V4, R1, R2, R3, and R4 growth stages, which was recorded to be 89, 86, 85, 83, 81, 82, 88, and 81 cm, respectively (Table 2.6). Height reductions were calculated as a percentage based on the heights collected from the untreated check plots. Height reductions were reduced to 8, 6, 10, 13, 9, 8, 13 and 6 % at the VE, V3, V4, R1, R2, R3, R4, and R5.5 growth stages, respectively (Table 2.6). The greatest height reduction was observed at the R1 growth stage, the same growth stage where the greatest amount of visual injury was observed.

Yield was reduced where applications were made at the VE, V3, V4, R1, R2, R3, and R4 growth stages, which resulted in 3387, 3281, 2711, 3146, 3503, 3293, and 3564 kg/ha, respectively as compared to 4175 kg/ha for the untreated check (Table 2.6). Yield reductions were calculated as a percentage based on the yield from the untreated check plots. Yield reductions were significantly higher at the VE, V3, V4, R1, and R3 growth stages; resulting in 16, 21, 27, 18 and 17% yield reductions, respectively. Greatest yield reductions were observed at the V4 growth stage, resulting in a 27% yield loss. No significant yield reductions were observed after the applications were made at the R4 growth stage, with the exception of the R5.5 growth stage.

These data indicate that 2,4-D applied to soybeans at different application timings can result in the greatest amount of visual injury and yield reduction at the V4 and R1 growth stages. These application timings correspond with the late vegetative and early reproductive growth stages. Soybean exposed to 2,4-D during these growth stages results in a significant yield loss. Based on these data, it can be determined that soybean growth stage is indeed an important factor when determining how severe a case of accidental drift or tank contamination of 2,4-D can potentially be to soybean yield loss.

In summary, these data depicted in this chapter indicate that in a situation of particle drift, tank contamination, or volatilization 2,4-D will not be as injurious to soybeans as other modes of actions may be. However, producers should still take caution when making applications of 2,4-D when susceptible crops are nearby. The potential for damage is still there; it would just take a greater amount of the herbicide to cause a significant yield loss when compared to other herbicides.

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	Population
2012	Starkville	May 15	AG 4932	140,000 seeds/ac
2012	Brooksville	May 1	AG 4932	140,000 seeds/ac
2013[b]	Starkville	May 30	PKP 95Y61	138,000 seeds/ac
2013[b]	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] 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.

Table 2.2 Visual ratings at 7, 14, 21, and 28 DAT for the effect of 2,4-D application timing and rate effects 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	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

Table 2.3 Height and height reductions from 2,4-D application timing and rate effects on soybean growth and yield[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 timing
[c] 1X application rate equivalent to 0.56kg ai/ha
[d] untreated check treatments

Table 2.4 Yield and yield reductions from 2,4-D application timing and rate effects on soybean growth and yield[a]

	Yield		Yield Reduction	
	Growth Stage		Growth Stage	
Rate[c]	V3[b]	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 timing
[c] 1X application rate equivalent to 0.56kg ai/ha
[d] untreated check treatments

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

	Height	Height Reduction[b]	Yield	Yield Reduction[b]
Growth Stage[c]	-----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] percent calculated from comparison of untreated check
[c] all application timings received 0.14 kg ae/ha of 2,4-D

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CHAPTER III

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

Abstract

With new crops containing auxin resistant traits being commercially sold in our near future, we can expect producers to gain many production benefits. Additional precautions will have to be made by producers to ensure they do not injure susceptible crop and non-crop species. Spray drift, contaminated spray equipment, and volatility from applications applied to other crops are some concerns that could be very injurious 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 a 1X (0.56 kg ae/ha), 1/4X, 1/16X, 1/256X, 1/1024X, and 0X rate 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 were significant. 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 R1 application timing resulted in a 46 and 41% yield reduction from the V3 and R1 application timings, respectively. The most sensitive growth stage of soybeans to dicamba was found to be the V4, V5, and R1 growth stages with 42, 45, and 38% injury. No significant visual injury or height reductions were recorded 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; 40, 51, 46, and 41%, respectively.

Nomenclature

Dicamba; 3,6 dichlor-2 methoxy benzoic acid; soybeans, Glycine max (L.) Merr.;

Abbreviations

MOA, mode of action; g ae/ha, grams acid equivalent per hectare; L/ha, liters per hectare; m, meter; kg ae/ha, kilograms of acid equivalent per hectare; DAT, days after treatment; cm, centimeter; bu/A, bushels per acre; WAE, weeks after emergence

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 result in crop injury (Egan et al. 2014). Symptomology observed from dicamba is typical of that of most auxin herbicides; epinastic twisting of the stems and petioles, cupping of the leaves, and swelling of the stems are some symptoms observed from dicamba on broadleaf plants species. All of these symptoms can be followed by chlorosis, inhibition of growth, wilting, and necrosis (Senseman 2007). The extent of symptomology that can be observed from dicamba exposure can be highly dependent on the amount of dicamba to which the plant has been exposed to.

Dicamba has been used for weed control for over fifty years, being one of the earliest used herbicides in our history. Throughout this time of use, little resistance to the herbicide has been recorded. With little weed resistance occurring over the many decades that dicamba has been used for weed control, it is unlikely that an acceleration in dicamba resistant weed species will occur like we have witnessed with the overreliance of 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 technology works because of the insertion of a gene that allows the plants to metabolize dicamba (Nandula 2010; Johnson, W. et al. 2012).

This gene was derived from the soil bacterium, *Stenotrophomonas maltophilla*, in order to develop the dicamba resistant gene (Nandula, 2010). With the use of dicamba in this tolerant cropping system, producers will receive a multitude of advantages. Multiple chemistries can be used for broadleaf weed control with this new cropping system and it will allow for overall improved weed control, especially in efforts to control glyphosate resistant weeds; all while reducing the risk of developing additional weed resistance by using a diverse selection of herbicides (Nandula 2012).

These new technologies offer many advantages, but with these advantages come many challenges that must not be overlooked. 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 there also should be concern with the herbicide volatilizing to off target application areas (Strachan et al. 2013). If proper application practices and good stewardship amongst one another 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 do so with great caution to prevent damage from occurring to their own or neighboring susceptible crops or non-crop plant species.

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 can result in extreme yield losses (Johnson,

V. et al. 2012; Wax et al. 1969; Kelley et al. 2005), depending on the rate to which has been applied to the soybeans. 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 visual 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 that has been observed where soybeans have been exposed to dicamba could be a moderate 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 could be more difficult tool to utilize; visual injury is subjective and could greatly vary depending on the individual's perception making the 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.

When soybeans are exposed to dicamba it is important to consider what growth stage those soybeans were in when the exposure occurred. In a study conducted by Griffin et al. (2013), dicamba applications were made in both the V4 and R1 growth stages with the greatest yield loss occurring from the applications that were made in the R1 growth stage. Another similar study applied dicamba at the V2, V5 and R2 growth stages and greatest yield losses occurred at the early bloom growth stage (R2) (Wax et al. 1969). Weidenhamer et al. (1989) applied dicamba at pre-bloom and mid-bloom growth stages and minimal yield differences were found in either application timing; this minimal difference could have been due to a later application of the pre-bloom timings. It is evident that soybean growth stage is a factor that must be taken into consideration when trying to determine as to how much yield loss will occur due to exposure to soybeans. Therefore, it is important to consider growth stage when evaluating a misapplication or accidental exposure of dicamba to soybeans.

Materials and Methods

During the growing season of 2012 and 2013 multiple experiments were conducted over four locations in the southeastern United States. These experiments were conducted to determine the effect of dicamba application rate and timing to soybean growth and yield. All experiments were conducted on 3.9 m wide by 12.2 m long plots (equivalent to four rows when on 38 inch 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 across other treatments. Each experiment conducted had four replicates at each location. The diglycolamine salt of dicamba was used for all objective discussed in this chapter.

Dicamba application rate and timing effect on soybean growth and yield

Experiments were conducted during the growing season of 2012 and 2013 to evaluate the effect of dicamba application timing and rate on soybean growth and yield. Experimental tests were conducted in the following 6 locations: 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 in Rohwer, AR (2013). Planting date, planting populations, 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 the application timing. Treatments were applied at two application timings, one at the V3 growth stage and the other at the R1 growth stage. Factor B consisted of the rate of dicamba applied. Application rates were based off a 1X rate of dicamba that was 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, 1/256X, 1/1024X titration corresponded to 0.56, 0.14, 0.0035, 0.00875, 0.00219, and 0.00055 kg ae/ha. The study also contained untreated check plots at all locations for comparison purposes. All treatments were applied using a two row (1.9m 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 as 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 7, 14, 21, and 28 DAT. Visual evaluations were collected as a percentage, ranging from 0 (0= no injury) to 100 (100= plant death), of overall soybean injury. Visual evaluations were collected at all locations with the exception of the Rohwer location. Plant heights were collected at the end of the growing season at all locations with the exception of the Rohwer location. Six plant heights were collected from each plot to obtain plant height data. Yield data were also 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 during the growing seasons of 2012 and 2013 to evaluate the effect of dicamba application timing on soybean growth and yield. Experiments were conducted in the following 6 locations: 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 in AR (2013). Planting date and planting populations 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; each additional application was made at weekly intervals until the plants began to naturally senesce. Growth stages of soybeans were carefully determined at each weekly application in order to evaluate at which growth stage soybeans are 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 as 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 7, 14, 21, and 28 DAT. Visual evaluations were collected as a percentage, ranging from 0 (0= no injury) to 100 (100= plant death), of overall soybean injury. Visual evaluations were collected at all locations with the exception of the Rohwer location. Plant heights were collected at the end of the growing season at all locations with the exception of the Rohwer location. Six plant heights were collected in each experimental unit in order to obtain plant height data. Yield data were also collected from the treated area using a mechanical harvester. Data were combined over all locations, analyzing location and year as a random effect. Data was 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 were expressed by rate and application timing. Visual injury was collected as a percentage and is displayed in Table 3.2. Visual injury ratings data collected 7 DAT was significant for all application rates at both application timings. The lowest application rate resulted in 7 DAT visual injury ratings of 12 and 16% at the V3 and R1 application timings, respectively. Visual injury 14 DAT was significant at all application rates and timings with injury ranging from 99 to 23%, depending on the rate and application timing. At 21 DAT visual injury that was observed ranged from 94 to 29%. Visual injury at 28 DAT was significant at application rate, timing, and the interaction of rate by timing. Injury from this rating date ranged from 98 to 27%, depending on application rate and timing. Overall visual injury increased over time; visual injury ratings were greater 28 DAT versus injury ratings observed at 7 DAT.

Application timing and application rate were significant factors with regards to plant heights (cm) and height reductions and no interactions were found. Plant height and height reductions data can be viewed in Table 3.3. Plant height data were pooled over all site years and application timings. Significant plant height were recorded at all application rates, plant heights ranged from 17 cm at the 1X rate to 88 cm at the 1/1024X rate of dicamba, as compared to 99 cm for the untreated. Height reductions were calculated as a percentage based on plant heights collected from the untreated check plots. It was determined that significant height reductions were observed at all application rates; with 1X rate resulting in a 92% height reduction and the lowest application rate, 1/1024X rate, resulting in a 11% height reduction. Plant height data were analyzed and pooled over all site years and application rates (Table 3.4). Plant heights that were collected from the V3 application timing were found to 66cm tall, and those that received dicamba applications in the R1 application timing were found to be 61 cm tall. Greater height reductions were observed from the R1 application timings, resulting in a 41% height reduction as compared to 34% in the V3 growth stage.

Application timing and rate were significant factors with regards to yield (kg/ha) and yield reductions and no interactions were found. 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 significant at all application rates with the exception of the lowest applications rate, 1/1024X. Yields data collected for 1X, 1/4X, 1/16X, 1/64X, 1/256X, and 1/1024X 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 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 observed for 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 from the soybeans that received applications in the V3 growth stage were found to be 2141 kg/ha as compared to 1990 kg/ha for treatments in the R1 growth stage. Yield reductions when pooled over location and application rate were significantly higher for the treatment that received the application in the R1 growth stage (46%), verses those that received the applications in the V3 growth stage (41%).

These data indicate that over time, injury from dicamba can increase the amount of injury observed. Also, it can be said that greater rates of dicamba result in greater visual injury, height reductions, and yield reductions. Where greater injury was observed we also observed greater yield reductions. 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

Growth stage at each weekly application timings was significant at all visual injury rating dates, plant heights (cm), height reductions, yield (kg/ha), and yield reductions. Visual injury ratings are displayed in Table 3.5, and plant height and yield data can be viewed in Table 3.6. Visual injury data were significant 7 DAT for all application intervals. The greatest visual injury observed at 7 DAT was at the V1 growth stage, resulting in visual injury rating of 37%. Visual injury ratings observed 14 DAT were significant at 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 data collected 21 DAT was significant at the VE through R4 growth stages. Visual injury ratings were greatest at applications made at the V1 through R1 growth stages (37, 40, 41, 38, 44, 34, and 37% visual injury, respectively). Visual injury 28 DAT was significant at the V1 through R1 growth stages; with the greatest amount of injury occurring at the V4, V5, V6, and R1 growth stages (42, 45, 38, and 40% visual injury, respectively).

Plant height (cm) and percent height reductions, calculated based on the untreated check plots, were both collected for this experiment (Table 3.6). Plant heights were found to be significantly shorter from 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 as compared to 101 cm for the untreated check. Significant plant height reductions were observed at the VE through R4 growth stages; 16, 11, 10, 20, 30, 34, 43, 30, 18, and 8%, respectively. The greatest height reductions were observed at the V5 and R1 growth stages resulting in height reductions of 34 and 43%; corresponding with 5 and 6 WAE applications.

Yield was collected and yield reductions were calculated as a percentage based on the yield from untreated check plots (Table 3.6). Yield was found to be significantly lower at the VE through R4 growth stages. Yields ranged from 3828 to 1906 kg/ha, depending on application timing. Percent yield reductions were found to be significant where applications were made at the V2 though R4 growth stages. Greatest yield reductions were found to have occurred at treatments receiving applications at the V4, V6, and R1 growth stages, resulting in a 40, 51 and 46% yield loss, respectively. These particular application timings correspond with the late vegetative and early reproductive soybean growth stages.

These data indicate that, like the previous experiment, dicamba injury increases over time. Plant height reduction was found to be a good indicator of yield reduction; where the greatest height reduction was observed the greatest yield losses also occurred. Soybeans were found to be 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 recorded after soybeans were in the R5 growth stage (the 8 WAE weekly application timing); which corresponds with the beginning of pod fill (Fehr and Caviness 1977). Based on these data it can be determined that soybean exposed to dicamba during pod fill can result in a 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 depicted in this chapter indicates that in a situation of particle drift, tank contamination, or volatilization dicamba can be detrimental to a soybean crop. 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 a late vegetative or early reproductive growth stage is likely to result in the greatest yield losses. With all of this in mind, it is crucial that great caution must be made when making applications of dicamba with nearby sensitive crops.

Table 3.1 Planting year, location, date, populations, and seed variety information for dicamba application rate and timing effect on soybeans growth and yield and for the dicamba application timing experiment

Year	Location	Planting Date	Variety	Population
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]

Growth Stage[b]	Days After Treatment			
	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

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