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Dicamba Effects on Soybean (Glycine max) Growth, Yield, and Offspring

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Dicamba Effects on Soybean (*Glycine max*) Growth, Yield, and Offspring

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ABSTRACT

Introduction of a new dicamba-resistant (Xtend) trait in soybean and cotton will increase dicamba herbicide use. Consequently, concern for injury to sensitive crops from off-target movement and tank contamination will likely increase. For soybean, foliar symptoms associated with dicamba damage do not necessarily reflect yield losses; hence, experiments were conducted to determine the effects of dicamba on soybean growth, yield, and offspring. Low rates of dicamba [1/64X (8.75 g ae ha^{-1}) and 1/256X (2.18 g ae ha^{-1}) of a normal 1X field rate (560 g ae ha^{-1})] were applied at two vegetative growth stages (V4, V6) and at each reproductive growth stage from R1 to R6. Compared to the nontreated check, dicamba applied during late vegetative and early reproductive growth of soybean caused leaf injury, plant height reduction, and yield loss. Regardless of soybean cultivar, the higher rate of 1/64X resulted in greater yield loss, with R1 being the most sensitive growth stage. Dicamba at 8.75 g ha^{-1} applied at R1 reduced mature soybean height 35 cm for an indeterminate cultivar and 23 cm for a determinate cultivar. Grain yield was reduced 14% for the indeterminate cultivar and 19% for the determinate cultivar. Injury and height reductions were less apparent when dicamba was applied during later growth stages. Offspring response to dicamba applied to parent plants the previous year was dependent upon application timing and dicamba rate. Negative effects to offspring were observed as reduced seed germination, seedling emergence, plant height, seedling vigor, pod malformation, and grain yield. Offspring had 17 to 23% injury when parent plants were treated from R4 to R6 with dicamba at 2.18 g ha^{-1}. Seeds from the bottom of the plant were affected more by dicamba than seeds from the top of the plant; however, no relationship existed between grain yield and pod malformation. This research shows that soybean cultivar, growth habit, or planting date may influence soybean recovery from dicamba.

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CHAPTER 1: LITERATURE REVIEW

Resistance of soybean to dicamba through trait introgression will provide growers an alternative weed management option, but the risk of injury to non-target crops from herbicide movement and tank contamination is of concern.

Glyphosate-Resistant Crops. Roundup Ready® technology (Monsanto Company, St. Louis, MO 63167) was introduced to crops in the mid-1990s, which allowed for the application of the non-selective herbicide, glyphosate over-the-top of cultivars containing the trait. Glyphosate-resistant soybean allowed growers to effectively manage difficult-to-control weeds with glyphosate, which was not possible prior to commercialization of the Roundup Ready technology. Unfortunately, the use of glyphosate over numerous cropping cycles has caused some weeds to evolve resistance to this herbicide (Heap 2014). Glyphosate effectively removed certain weeds while other less sensitive ones became prevalent and problematic (Beckie et al. 2000). The long-term sole use of a single herbicide is the source of current resistance problems that farmers across the U.S. are dealing with daily.

Technology Advancements of Glyphosate-Resistant Crops. In response to this resistance problem, advances in genetic engineering have led companies to develop crop cultivars with resistance to additional herbicide modes of action. In 2015, Monsanto released the Roundup Ready® Xtend Crop System (Monsanto Company, St. Louis, MO 63167), which is a new technology that will allow the use of both dicamba and glyphosate in soybean and dicamba, glyphosate, and glufosinate in cotton (*Gossypium hirsutum* L.) (Seifert-Higgnins and Arnevik 2012). The Roundup® Xtend herbicide, a premix of glyphosate and the diglycolamine (DGA) salt of dicamba with a polybasic polymer added to reduce dicamba volatility, will be available for preplant application and in-season over-the-top use in both of the trait-containing crops when approved by the Environmental Protection Agency. The DGA salt of dicamba will also be sold separately to allow growers flexibility in use and in selection with glyphosate formulations (Monsanto Company, St. Louis, MO 63167). Engenia™ herbicide (BASF Corporation, Research Triangle Park, NC 27709) is a dicamba product {BAPMA [N,N-Bis-(aminopropyl)methylamine]} formulated as a tridentate amine salt, that has lower volatility than the DGA salt formulations (Xu et al. 2012). Engenia is available for use in the Roundup Ready® Xtend Crop System. Monsanto and BASF claim to have developed formulations that will reduce the potential for dicamba movement through volatilization.

Dicamba. Dicamba (3,6-dichloro-2-methoxybenzoic acid) is a benzoic acid herbicide that was discovered by S.B. Richter in 1958 (Senseman 2007). Having a similar chemical structure and mode of action to phenoxy herbicides, dicamba mimics auxins, a type of plant hormone, and causes abnormal growth by affecting cell division (Cremlyn 1991). Dicamba has been used for more than five decades to control a wide range of dicotyledonous weeds in corn (*Zea mays* L.), small grains, and pasture (Egan and Mortensen 2012). Although several different forms of dicamba are used as herbicides, the dimethylamine salt and the sodium salt are the most common. When looking at herbicide movement in plants and in the atmosphere, chemical properties of each formulation are important. Dicamba has a vapor pressure of 4.5×10^{-3} Pa (25 C°) and water solubility of 4,500 mg L^{-1} for the acid, 720,000 mg L^{-1} for the dimethylamine (DMA) salt, and 400,000 mg L^{-1} for the sodium salt. Compared to 2,4-D, dicamba penetrates plant tissue slightly less rapidly, and among formulations, the DMA salt penetrates leaf foliage more rapidly than other formulations (Andersen et al. 2004). Dicamba is sold under many trade names including Banvel® (Arysta LifeScience North America LLC, Cary, NC 27513) and Clarity® (BASF Corporation, Research Triangle Park, NC 27709).

Off-target Herbicide Movement. An increase in occurrences of off-site movement onto neighboring sensitive crop fields is likely to occur with the commercialization of the Roundup Ready® Xtend Crop System (Monsanto Company, St. Louis, MO 63167) (Andersen et al. 2004). Physical particle drift, volatilization, and tank contamination are the main sources for off-target movement.

All herbicides are prone to off-target movement as spray particles can drift. Spray droplet size, wind speed, nozzle selection, and boom height above the intended target are primary contributors to herbicide drift (Hatterman-Valenti et al. 1995). Herbicide drift is most often the result of improper application (Wauchope et al. 1982). Between 1 to 8% of the spray solution with ground sprayers typically moves via physical drift beyond the spray swath, depending on nozzle type and wind speed, and 20 to 35% drift with aircraft spraying (Maybank et al. 1978). Physical drift of droplets is largely dependent on the mechanical properties of the sprayer. Spray droplets that can drift can be reduced from 30 percent down to 2 percent by simply upgrading the nozzle technology in current spray setups (Ramsdale and Messersmith 2001). One of the primary factors influencing physical drift include droplet size and dissemination, predominantly when herbicides are applied as ultra-low volume sprays where spray droplets are less than 105 microns in size (Hanks 1995). Droplet drift can be reduced with proper nozzle selection, appropriate pressure, and with the use of drift retardants, which are designed to increase droplet size (Hanks 1995). With the new formulations of dicamba, off-target movement resulting from volatilization is less likely than the older formulations; albeit, physical particle drift of these new products is possible (Robinson et al. 2013).

There is a possibility for auxin herbicides, such as dicamba to move off-site through volatilization alone and subsequent vapor drift of the chemical. Volatilization is the conversion of a substance from a liquid or solid to a gas state. All herbicide formulations volatize; however, the rate of volatilization depends on the vapor pressure of the chemical. High vapor pressure results in greater volatility of the formulation. Differences in physical or chemical properties of the leaf surface of a plant and to environmental factors such as temperature, rainfall, and relative humidity can facilitate herbicide volatility (Egan and Mortenson 2012). For most herbicides, the vapor pressure is low enough that volatility is of little consequence. For other herbicides, however, volatility can result in significant off-target movement. Behrens and Lueschen (1979) stated that dicamba volatilization occurred from treated corn and was detected on soybean 3 days after the application and soybean injury was detected up to 60 m downwind of treated corn. Using field studies and growth chambers, it was determined that lowering temperature or increasing relative humidity can reduce dicamba volatility effects on soybean (Behrens and Lueschen 1979; Grover 1975).

Tank contamination can also have a detrimental effect on sensitive plants. Tank contamination can occur from herbicide residues in spray-contaminated tanks, nurse tanks, transfer hoses, screens, measuring containers, or jugs. Boerboom (2004) reported that spray equipment cleaned with an ammonium-water solution after dicamba application had up to 0.63% of the dicamba use rate exiting from the sprayer on the next application. Dicamba is a difficult herbicide to remove from spray tanks and multiple rinses with ammonia are vital when dicamba is used (Boerboom 2004).

Soybean Response to Dicamba. In dicamba-resistant soybean, approved dicamba products can be applied pre-plant, at planting (PRE), and postemergence (POST) (Seifert-Higgins and Arnevik 2012). With a wide range of applications during the growing season and considering a wide range of planting dates, there is an expected increase in the opportunity for off-target movement. Injury from the exposure of dicamba on soybean plants is normally noticeable as foliar symptoms. These symptoms can take up to a week to manifest; however, they are usually expressed by leaf cupping, plant malformation, and reduced growth (Wax et al. 1969). Research has been conducted to evaluate the extent of dicamba injury on soybean. Dicamba applications during early vegetative growth affects the new leaf development but does not affect pod and seed production (Auch and Arnold 1978). Al-Khatib and Peterson (1999) reported that initial symptoms of severe petiole epinasty and leaf curling on soybean were observed within 3 hours after dicamba treatment at the highest rate (186.6 g ha^{-1}) and 1 day after treatment (DAT) for the lowest rate (5.6 g ha^{-1}) (1/100 to 1/3 the use rate of 560 g ha^{-1}). Seven days after dicamba treatment at the V3 stage of soybean, treated plants at the high rate were injured an average of 66%, and by 14 DAT, injury was 92%. Dicamba at the low rate reduced soybean height 15%, but yield was reduced only 2% whereas the high rate reduced soybean height 75% and yield was reduced 45%. In two experiments, Andersen et al. (2004) reported 30 to 40% soybean injury 7 DAT from a V3 application of dicamba at a rate of only 5.6 g ha^{-1} and 80% injury at 186.6 g ha^{-1}. Soybean yield was reduced 14 to 34% from dicamba at the low rate and 72 to 83% at the high rate. Johnson et al. (2012) reported soybean injury 7 DAT following dicamba applied at 3 g ha^{-1} pre-bloom (20- to 30-cm soybean/V5) of 8 to 21% and a yield loss ranging from 1 to 20%. Injury to soybean from dicamba applied at 41 g ha^{-1} was 37 to 80% with a yield loss of 13 to 85%. Variability among results has led most scientists to the conclusion that visible estimates of dicamba injury during vegetative growth are a moderate indicator of yield response.

Dicamba applications during reproductive growth stages have also been examined. Wax et al. (1969) made applications to soybean at mid-bloom and reported a yield reduction of 23% when dicamba was applied at 4.4 g ha^{-1} and a 75% yield reduction at 35 g ha^{-1} at the same growth stage. Auch and Arnold (1978) applied dicamba at early bloom and reported that dicamba at 11 g ha^{-1} reduced soybean yield 34 to 42% and 56 g ha^{-1} reduced yield 36 to 67%.

Soybean injury and yield responses to dicamba applied at vegetative and reproductive growth stages are difficult to predict. At a dicamba rate of 11 g ha^{-1}, an insignificant yield loss occurred from a V1 application, an 8% increase resulted from a V2/V3 application, and a yield decrease of 9% followed an early-bloom treatment (Auch and Arnold 1978). However, in a separate study, dicamba applied at 8.75 g ha^{-1} resulted in a yield loss of 4% when it was applied to pre-bloom soybean and a loss of 23% when applied at mid-bloom (Wax et al. 1969). In an additional experiment, soybean yield was greater following mid-bloom applications than pre-bloom applications of dicamba at 5 g ha^{-1} (Weidenhamer et al. 1989), and no effect on yield was seen for application of 5.6 g ha^{-1} at the V3 and R2 growth stages (Kelley et al. 2005). Much of the unpredictable yield loss has been attributed to application timing, dicamba rate, and environmental conditions (Andersen et al. 2004; Auch and Arnold, 1978; Weidenhamer et. al 1989), but the plasticity of soybean makes yield differences difficult to explain.

There is a clear understanding that dicamba injury can have detrimental effects on soybean growth and yield; however, undetermined factors may also influence yield. For example, an obvious visual symptom of dicamba drift is reduction in height; however, one cannot always expect yield reduction when height is reduced (Auch and Arnold 1978). Weidenhamer et al. (1989) reported that in a drought-stressed year, as little as 0.4 g ha^{-1} of dicamba applied at R1 caused a 10% yield loss whereas a year with adequate rainfall, the same yield loss was seen at a rate of 15 g ha^{-1}. Drought stress has the potential to inhibit the ability of soybean to metabolize dicamba, which allows it to stay active in the plant for a longer period of time, which results in the abortion of flowers and pods (Robinson et al. 2013). Drought would also limit the degree of symptomology exhibited by the plant. The variation of conditions complicates the understanding of dicamba on soybean and a transparent conclusion on dicamba drift is difficult across different geographies.

Soybean Offspring Response to Dicamba. Dicamba has the potential of being stored in the seeds of the soybean plant. The movement of this herbicide in the plant is similar to the transport of photosynthate. When photosynthate is stored in the seed, dicamba may be stored in the seed as well (Thompson and Egli 1973). Herbicides stored in soybean seed can decrease germination and can be injurious to the developing seedling (Wax et al. 1969). Solomon and Bradley (2014) examined the influence of application timings of several synthetic auxin herbicides on soybean and determined that following a V3 application of dicamba at sub-lethal rates, the number of pods per plant was similar to that of the non-treated control. In contrast, following a R2 application, the number of pods per plant was highly influenced by herbicide rate. In general, applications of synthetic auxin herbicides made during reproductive growth stages could potentially result in dicamba carryover in the seed more than applications made at earlier growth stages.

Dicamba has the potential for severely restricting pod formation and seed production in soybean. Thompson and Egli (1973) found that plants treated with dicamba at 560 g ha^{-1} at flowering produced no seed. Seed production from plants treated with 220 g ha^{-1} at flowering and 560 g ha^{-1} at pod fill was significantly reduced. None of the seeds treated with dicamba at 30 or 220 g ha^{-1} at pod fill was classified as normal in a standard germination test, and at least 70% of the seeds were classified as "dead," with the remaining exhibiting abnormal germination (Thompson and Egli 1973). Only 50% of the seed from the top and 36% of the seed from the bottom of plants treated with dicamba at 30 g ha^{-1} at flowering germinated normally. Of the emerged seedlings, those from the top of parent plants treated at flowering and the pods from entire plants treated at pod filling were less vigorous than offspring of non-treated plants. Malformation seen in these off-spring seedlings varied from slight crinkling and downward curvature of the leaf margins to leaves that did not expand fully. The most severe injury was observed in plants treated during pod fill (R4) (Thompson and Egli 1973). Dicamba injury of the offspring was expressed mostly in the trifoliate leaflet; however, there was some injury to the unifoliate leaves when dicamba was applied to parent plants during flowering. No visible signs of injury were exhibited in second and third trifoliate leaves (Thompson and Egli 1973). Visible injury observed in soybean offspring following dicamba exposure may differ when treated with higher rates.

Previous research with dicamba has provided an overview of the potential risks associated with the herbicide. Moving forward with the Xtend technology in crops, will necessitate continued research to determine the correlation between soybean exposure to dicamba and expected yield. In addition, further research is needed to understand the effects of dicamba on soybean offspring. The implication that dicamba can be transported to the seed of susceptible cultivars could have significant impacts on producers who grow seed of conventional or other transgenic lines that are not resistant to the dicamba herbicide.

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CHAPTER 2: SOYBEAN RESPONSE TO DICAMBA: CULTIVAR, MATURITY, AND GROWTH HABIT

ABSTRACT

Introduction of the Roundup Ready® Xtend System will provide an alternative weed management option for growers, but the risk of dicamba injury to sensitive crops, particularly soybean from off-target movement and tank contamination, is of concern. Experiments were conducted to determine the response of soybean to low rates of dicamba over a wide range of application timings. Two glufosinate-resistant varieties (HBK 4950LL and HALO 5.45LL) commonly grown in Arkansas were chosen for these studies. Two rates of dicamba, 2.18 g ha^{-1} and 8.75 g ha^{-1} (1/256X and 1/64X of the postemergence labeled rate for dicamba-resistant soybean), were applied at two vegetative (V4, V6) and six reproductive (R1-R6) growth stages. Compared to the nontreated check, dicamba applied during late vegetative and early reproductive growth of soybean caused leaf injury, plant height reduction, and yield loss for both soybean cultivars. Averaged across dicamba rates applied at R1, soybean yield was reduced 14% for the HBK 4950LL cultivar and 19% for the HALO 5.45LL cultivar. Dicamba applied at R1 to the HALO 5.45LL and HBK 4950LL soybean resulted in 48% and 43% visible injury 4 weeks after treatment. Grain yield was similar to that of the nontreated when dicamba was applied at the later reproductive stages; however, pod malformation and pod loss were greater following an R4 application compared to other application timings. Furthermore, it was determined that soybean recovery differs depending upon soybean cultivar; however further research is needed to determine which is more influential: soybean maturity, growth habit or planting date.

Nomenclature: Dicamba; soybean, *Glycine max* (L.) Merr.

Key words: drift, tank contamination, progeny, crop injury

INTRODUCTION

As a result of the continuing increase of herbicide-resistant weeds, advances have been made in technology that has led to the development of dicamba-resistant soybean (Seifert-Higgins and Arnevik 2012). This new technology, referred to as the Roundup Ready® Xtend system (Monsanto Company, St. Louis, MO 63167), was recently approved and will offer growers an additional weed control option. The technology allows for the over-the-top use of both dicamba and glyphosate in soybean and dicamba, glyphosate, and glufosinate in cotton (*Gossypium hirsutum* L.). However, as with the release of the glyphosate-resistant cultivars (Dill 2008), an increase in dicamba herbicide use will result in a greater potential for off-target movement. Dicamba has been used historically for control of broadleaf weeds in corn (*Zea mays* L.) and is known for having the potential to travel from the intended target (Behrens and Lueschen 1979).

Injury to sensitive crops can occur via particle drift, volatility, or tank contamination, as well as other means. Anticipating potential problems, Monsanto Company and BASF (100 Park Ave., Florham Park, NJ 07932) have been working with growers, agricultural service providers, land grant universities, and university extension to develop stewardship practices for these technologies. These practices include the development of low volatility formulations of dicamba, adjuvants, and herbicide premixes that reduce drift, as well as advanced spray nozzle designs that limit fine spray droplets. In dicamba-resistant soybean, dicamba applications can be made pre-plant, at planting (PRE), and after the crop emerges (POST) (Seifert-Higgins and Arnevik 2012). With a wide range of applications during the growing season and a wide range of planting dates in the mid-South US, the opportunity for off-target movement is expected to increase (Barber et al. 2015; Norsworthy et al. 2015). Although symptomology resulting from synthetic auxin herbicides is easily recognized, subsequent yield loss would be dependent on the herbicide rate, specific crop, timing of application, and weather conditions prior to and following application (Griffin et al. 2013; Scholtes and Reynolds 2014).

Crop injury and yield loss of soybean in response to dicamba have been extensively researched. Common symptoms of off-target movement of synthetic auxin herbicides, including dicamba, on soybean include parallel venation and cupping of the leaf, stem and leaf epinasty, cracked and swollen stems, and eventually chlorosis, inhibition of growth, and necrosis (Al-Khatib and Peterson 1999; Andersen et al. 2004, Auch and Arnold 1978; Kelley et al. 2005, Sciumbato et al. 2004; Wax et al. 1969). Al-Khatib and Peterson (1999) reported that initial symptoms of severe petiole epinasty and leaf curling on soybean were observed within 3 hours after dicamba treatment at the highest evaluated rate (186.6 g ha^{-1}) and 1 day after treatment (DAT) for the lowest rate (5.6 g ha^{-1}) (1/100 to 1/3 of the use rate of 560 g ha^{-1}). The timing of the synthetic auxin herbicide exposure may have a significant impact on the severity of soybean height and yield reductions (Al-Khatib and Peterson 1999; Solomon and Bradley 2014). Al-Khatib and Peterson (1999) found that soybean plants treated at third trifoliate stage, V3 with dicamba at 187 g ha^{-1} expressed 66% injury 7 DAT and 92% injury 14 DAT, resulting in a height reduction of 75% and yield reduction of 80% when compared to the untreated check. When dicamba at 56 g ha^{-1} was applied at V3, a 45% yield reduction was observed (Al-Khatib and Peterson 1999). Andersen et al. (2004) in two studies applied dicamba at 5.6 g ha^{-1} at V3 and reported 30 to 40% soybean injury 7 DAT and yield reductions of 14 to 34% when compared to the untreated check. In a similar study, only 6% yield reduction was observed when dicamba at 5.6 g ha^{-1} was applied at V3 (Kelley et al. 2005). Andersen et al. (2004) reported 80% injury following an application of 187 g ha^{-1} at V3, resulting in yield loss of 72 to 83%. Johnson et al. (2012) reported soybean injury of 8 to 21% 7 DAT from dicamba applied at 3 g ha^{-1} pre-bloom and a yield loss of 1 to 20%. At 41 g ha^{-1}, injury to soybean from dicamba was 37 to 80% with a yield loss of 13 to 85%. Variability among the results leads to the conclusion that visible estimates of dicamba injury during vegetative growth are only a moderate indicator of yield response (Griffin et al. 2013).

Dicamba applications during soybean reproductive growth stages have also been examined. Greater injury and yield reductions occurred when dicamba was applied at later soybean growth stages (Auch and Arnold 1978; Wax et al. 1969). Dicamba applied to an indeterminate soybean at 17.5 g ha^{-1} at bloom reduced soybean plant height 46% and resulted in a yield loss of 52% when compared to the untreated check (Wax et al. 1969). Scholtes and Reynolds (2014) also applied dicamba at 17.5 g ha^{-1} at bloom and reported height reduction of 28% and yield reduction of 36%. At 11 g ha^{-1}, dicamba applied at early bloom (R1) reduced soybean yield 34 to 42% and 56 g ha^{-1} reduced yield 36 to 67% (Auch and Arnold 1978). Wax et al. (1969) applied dicamba to soybean at mid-bloom and reported a yield reduction of 23% when applied at 4.4 g ha^{-1} and 75% at 35 g ha^{-1}. Low rates of dicamba applied to soybean later in the growing season cause minimal effects on observable injury and yield. For instance, dicamba applied after R5 resulted in no significant observable injury, height reductions, or yield reductions in research conducted in the mid-South US (Scholtes and Reynolds 2014). Although Griffin et al. (2013) reported that soybean at flowering is 2.5 times more sensitive to dicamba compared with vegetative exposure in regards to yield loss, determining conclusive findings between estimates of injury and yield loss were difficult.

Soybean yield is a function of plant population, the number of seed produced per plant, and seed weight. Dicamba deleteriously affects each of these yield components (Weidenhamer 1989). Soybean plants exposed to dicamba become malformed and have altered morphology and reduced growth. Soybean plants exposed to low rates of dicamba during vegetative growth had stimulated lateral development and increased branching, especially when the apical meristem died (Andersen et al. 2004; Wax et al. 1969), but dicamba did not affect pod and seed production because the herbicide was likely detoxified before reproduction began (Auch and Arnold 1978; Solomon and Bradley 2014). The severity of leaf injury was influenced by application rate, but not growth stage. In contrast, it was determined that following a V3 application of dicamba at sub-lethal rates, the number of pods per plant was similar to that of the non-treated control, whereas following an R2 application, the number of pods per plant was highly influenced by herbicide rate (Solomon and Bradley 2014).

A subsequent consequence of soybean exposure to dicamba during flowering or early-pod production was abnormal pod formation (Auch and Arnold 1978; Kelley et al. 2005). A reduction in yield was observed due to limited pod production, in return reducing pod number, seed number, and seed weight (Kelley et al. 2005; Wax et al. 1969). Soybean yield reductions are likely more correlated with seeds per pod than pods per plant or seed weight (Solomon and Bradley 2014).

Conclusions can be made that soybean injury and yield loss following dicamba exposure is influenced by herbicide rate and growth stage during exposure; however, some research indicates that cultivar selection affects soybean recovery from herbicide injury (Weidenhamer 1989). A soybean cultivar is identified by its maturity group and growth habit. Early-maturing soybean tend to be classified as indeterminate while determinate soybean are normally later maturing. Wax et al. (1969) suggested that soybean yield response to dicamba at different growth stages may depend on whether soybean are determinate or indeterminate. Weidenhamer (1989) reported a greater negative effect on yield of indeterminate soybean exposed to dicamba at flowering than for determinate soybean that cease vegetative growth at the onset of flowering. Furthermore, the decreased amount of time between planting and flowering in early-maturing soybean reduce leaf area and may limit the opportunity to recover from early-season herbicide injury (Holshouser 2001). Determining the recovery of soybean based on growth habit and maturity may help to alleviate this seemingly unpredictable yield loss.

The objective of this research was to determine the response of soybean to low rates of dicamba. To investigate this objective, several trials were conducted using indeterminate and determinate soybean of various maturity groups.

MATERIALS AND METHODS

Field experiments were conducted in 2014 and 2015 at the Lon Mann Cotton Research Station in Marianna, Arkansas on a Calhoun silt loam soil and Rohwer Research Station in Rohwer, Arkansas on Sharkey clay soil. These experiments were conducted to determine the effect of dicamba application rate and timing on soybean growth, yield, and offspring. The DGA salt formulation of dicamba (Clarity® herbicide, BASF Corporation, Research Triangle Park, NC 27709) was used for this research. For all experiments, dicamba treatments were applied using an air-pressurized tractor-mounted sprayer calibrated to deliver 140 L ha^{-1} at 270 kPa. Sprayers were fitted with four 110 degree XR air-induced flat-fan nozzles (TeeJet Technologies, Springfield, IL 62703) spaced 41 cm apart. Applications were applied at wind speed of no more than 4.8 km h^{-1}. Soybean was planted on raised beds spaced 97 cm apart using a four-row planter. Each plot consisted of four rows and treatments were applied to the center two rows of each plot to avoid cross contamination between plots. Nontreated border areas between plots were 1.94 m wide. Cross contamination between adjacent treated plots was not observed during weekly visual inspections. Fertility, weed control, irrigation, and overall management practices implemented were research-based University of Arkansas Extension recommendations and were field and production system specific (Arkansas Soybean Production Hankbook-MP197).

Dicamba Application Timing and Rate Effect on Soybean Growth and Yield. Experiments were conducted on an HBK 4950 LL, an indeterminate soybean, at both locations in 2014 and 2015. HALO 5.45 LL, a determinate soybean was planted at Rohwer, AR in 2014 and Marianna, AR in both 2014 and 2015. Soybean varieties were evaluated in separate trials. An additional site was evaluated in Marianna, AR on the HBK 4950 LL soybean in 2015 at a later planting date. For experiments conducted at each location, soybean cultivar, maturity group, planting date, and harvest date information are provided in Table 1. Experiments were organized as a two-factor factorial, randomized complete block design, with four replications. Factor A was soybean growth stage and factor B was dicamba rate. Soybean was planted at 288,000 seeds ha^{-1}. The two low rates of dicamba evaluated included: 1/64x (8.75 g ha^{-1}) and 1/256x (2.18 g ha^{1}) of a normal rate (560 g ha^{-1}) for POST use in dicamba-resistant soybean. Applications were made at the V4 and V6 stages and at each reproductive stage starting with R1 and ending with R6. Experiments also included a nontreated check at all locations for comparison purposes. Applications were made within 5 days (+/-) of the intended growth stage depending on weather conditions.

Visual estimates of percent crop injury were recorded 1, 2, and 4 wks after treatment (WAT) on a scale of 0 to 100%, where 0 equals no injury and 100 was complete crop death. Soybean heights were evaluated by measuring five random soybean plants per plot from the soil surface to the top of the central stem or terminal. Heights were taken 2 WAT and at plant maturity. After plants reached full maturity, 1 m of row was collected from the center of each plot and used for additional measurements. Using the plant samples from the 1-m row, ten random plants were selected and pods were hand harvested from each plant. Data were collected on total pod number and percentage of pod malformation on each plant. Soybean was harvested from the center two treated rows of each plot with a small-plot combine, and seed yields were adjusted to 13% moisture content.

Data for visual estimates of injury, plant height, pod malformation, and soybean yield were subjected to ANOVA using JMP 11.0.0 (SAS® Institute Inc., Cary, NC 27513) to test for the significant effects of dicamba rate, treatment timing, and their interaction. Location and year combinations were considered an environment sampled at random, as suggested by Blouin et al. (2011). Considering year and location as random effects permits inferences about treatments to be made over a wide range of environments (Carmer et al. 1989). Dicamba rate and application timing were fixed effects in the model, whereas replications were random effects. Differences between treatments were based on Fisher's protected LSD at α=0.05. Although, means from nontreated plots were not included in the ANOVA, Tukeys HSD (Honest Significant Difference) test was used to find out which specific means are different from the nontreated and significant differences were denoted with an asterisk.

Influence of Soybean Growth Habit on Recovery Following Dicamba Application. In a separate experiment, three indeterminate and two determinate glufosinate-resistant varieties were evaluated to determine if soybean responds differently to low rates of dicamba based on growth habit. This experiment was conducted twice in 2015 in Marianna, Arkansas. Specific cultivars, date of application, growth habit, planting date, and harvest date information are provided in Table 3. The experiment was organized as a split-plot design with four replications. The main plot was growth habit and the sub plot was dicamba rate. Dicamba rates evaluated were similar to the previous experiment (2.18 g ha^{-1} and 8.75 g ha^{-1}) and were applied at the R1 growth stage of each cultivar. Planting and application techniques were similar as the previous experiment.

Visual estimates of injury were rated 2 WAT and plant heights were collected 3 WAT. Soybean plants were harvested from the center two rows of each plot with a small-plot combine, and seed yields were adjusted to 13% moisture content.

Data for visual estimates of injury, plant height, and soybean yield were subjected to ANOVA using JMP 11.0.0 (SAS® Institute Inc., Cary, NC 27513) to test for the significant effects of growth habit and dicamba rate. Each planting date was analyzed separately. Growth habit and dicamba rate were considered fixed effects in the model, whereas replications were considered random effects. Soybean cultivar was nested within growth habit. In this sort of design, each soybean cultivar is given a unique identity because it is not replicated across a treatment. It is unique to that particular treatment because of its growth habit. Analyses were performed on the least square means and detected using Fisher's protected LSD at α=0.05.

RESULTS AND DISCUSSION

Symptomology observed for dicamba consisted of chlorosis of terminals, cupping and crinkling of uppermost leaves, swollen petiole bases, and stem and leaf epinasty. Severity of leaf injury in this study was influenced by dicamba rate and application timing.

HBK 4950LL Soybean. A significant interaction of application timing and dicamba rate resulted for visual injury of the HBK 4950LL soybean 1 and 2 WAT; however, no interaction was observed 4 WAT. From general observations, dependent upon dicamba rate, injury resulting from dicamba applied early in the growing season, prior to beginning pod (R3), became visible within a week of application and tended to become more severe within 2 WAT. Once soybean plants began to set pods, injury was less severe and within a week of application injury was nearly unidentifiable. Plants treated with dicamba at 8.75 g ha^{-1} at V4 were injured 24% 1 WAT and increased to 37% 2 WAT (Table 4). Averaged over both dicamba rates, the most severe injury was observed from applications made at V6 and R1 stages, resulting in 42 and 43% injury as late as 4 WAT (Table 4). Visual injury observed at each application timings was influenced by herbicide rate at 1 and 2 WAT; however, at 4 WAT, only a 4% difference in injury was observed between the two rates when averaged across all application timings (Table 5).

Reductions of plant height resulting from dicamba applications were dependent upon the main factors of application timing and dicamba rate. Height reductions were observed at 2 WAT for applications made prior to R3 and plants remained stunted throughout the season. As with visual injury, the amount of height reduction increased as dicamba was applied at earlier growth stages. Averaged across the two rates, the most severe stunting was observed following an R1 application, resulting in an average plant height reduction of 35% when compared to the nontreated (101 cm). Similar results were seen in plant height following applications made at V4, V6, and R2 applications (Table 4). Furthermore, dicamba applications resulting in height reduction compared to nontreated soybean also resulted in yield loss. Although, dicamba rate did not interact with application timings for stunting, dicamba applied at 8.75 g ha^{-1} reduced heights 23% 2 WAT, and 14% reduction resulted following dicamba at 2.18 g ha^{-1}.

Dicamba rate and application timing each had a significant effect on grain yield of the HBK 4950LL soybean; however, no interaction was observed. As stated in previous literature, results indicate that when dicamba is applied during flowering (R1) soybean is highly susceptible to yield loss (Auch and Arnold 1978; Wax et al. 1969). Averaged across dicamba rate, an R1 application resulted in a 14% yield loss, whereas dicamba applied at R5 or R6 yielded within 3 to 4% of the nontreated (Table 4). It was also found that dicamba applied at R3 and R4 reduced soybean yield 7 and 6% when compared to the nontreated (Table 4). When averaged across application timings, relative yield differed 2% between the two rates (92% and 94%) (Table 5).

Dicamba applied during flowering or early-pod production caused abnormal pod formation, resulting in fewer pods produced; however, there was no relationship of pod number and grain yield. Yield loss following dicamba injury may be more associated with other yield components, such as seed per pod and/or weight of that seed (Kelley et al. 2005, Solomon and Bradley 2015).

HALO 5.45LL Soybean. An interaction of application timing and dicamba rate was observed on visual injury of the determinate HALO 5.45LL soybean 1 and 4 WAT, but not at 2 WAT (Table 6). At each application timing, injury observed 1 WAT between the two rates differed following dicamba applications at V4, V6, R3, and R4. Three weeks later, differences in injury were distinguishable following V4, V6, and R1 applications. Similar to results seen in the HBK 4950LL soybean cultivar, injury symptoms resulting from dicamba applications made during late vegetative and early reproductive growth appeared 1 WAT and tended to increase over the next 2 to 4 weeks. Observable leaf injury to soybean was reduced when dicamba was applied after the R3 growth stage. At 2 WAT, the greatest injury, ranging from 27 to 44 %, followed applications made during late vegetative/early reproductive growth stages. By 4 WAT, soybean injury increased to 55% following dicamba at 8.75 g ha^{-1} applied at V6, similar to the 48% injury from 8.75 g ha^{-1} applied at R1 (Table 6).

No interaction between application timing and dicamba rate was observed on plant height 2 WAT and at plant maturity. Once soybean reached maturity, plant height was reduced more from dicamba applied at V6 and R1 than at other application timings. Compared to the nontreated check, a 40% height reduction was observed 2 weeks following a V6 application (data not shown) and remained 33% stunted at maturity (Table 6). Following an R1 application, a 35% height reduction was observed 2 WAT (data not shown) and at maturity soybean height was 29% less than the nontreated (Table 6). At maturity, plant heights were similar following V4 and R2 dicamba applications, ranging from 22 to 29% reduction when compared to the nontreated (89 cm) (Table 6). Similar to the HBK 4950LL soybean, a significant height reduction observed at plant maturity resulted in a significant yield loss. When applied after R2, the effect dicamba had on plant height became less apparent (< 10% reduction in height) (Table 6). At plant maturity, soybean height was reduced slightly less with dicamba applied at 2.18 g ha^{-1} than with the 8.75 g ha^{-1} rate (13 and 17%, respectively) (Table 7).

A significant yield reduction in HALO 5.45LL, a determinate soybean, depended on application timing. On average, 19% yield loss occurred following an R1 application; however, yields recorded from V4, V6, and R2 treatments were statistically similar (Table 6). When compared to the nontreated check, no significant yield loss was recorded from applications after R3. Relative yields of 97 to 99% were collected from R3 and R5 applications (Table 6). Relative yields from 90 to 95% resulted from dicamba applications at R4 and R6 (Table 6). Averaged across application timings, yield loss in the determinate soybean was reduced almost half by dicamba at 2.18 g ha^{-1} compared to 8.75 g ha^{-1} (Table 7). Similarly to the HBK 4950LL soybean, dicamba applied during flowering and pod fill caused abnormal pod formation and restricted pod fill; however, neither parameter was related with grain yield (data not shown). Following dicamba exposure, soybean has the ability to produce new axillary buds, eventually resulting in new flowers and seed pods (Moore 1979); however, determinate or indeterminate, it has been shown in previous research that the effect of dicamba on soybean yield is influenced by seed number or seed weight more so than pod number.

Soybean Growth Habit. A preliminary field trial in 2014 was used to evaluate soybean cultivar response to dicamba (data not shown). Due to the unexplainable differences in yield, an experiment was designed and conducted twice in 2015 to determine if growth habit had an effect on soybean recovery following dicamba exposure. Dicamba was applied to several soybean cultivars at the R1 growth stage. Soybean cultivars, their growth habit, and application date can be found in Table 3. Due to more than one month difference in planting dates between the two trials, planting dates were analyzed separately (Table 8).

For both planting dates (13 May 2015 and 1 July 2015), there was no interaction of the two factors, but growth habit and dicamba rate, each factor had a significant effect on visual injury, plant heights, and grain yield. (Table 8). Following an R1 application of dicamba, visual injury and height reduction for the first planting date were greater for soybean of indeterminate growth; however, relative yield did not differ between indeterminate and determinate growth habits (Table 9). Plant heights of soybean cultivars of indeterminate growth were reduced 43 to 53% with relative yields of 73 to 85% of the nontreated check; whereas for the determinate varieties, height reductions ranged from 33 to 36% with relative yields of 79 to 80% (Table 10). Regardless of growth habit, dicamba at 2.18 g ha^{-1} applied to soybean resulted in 34% injury 2 WAT and a height reduction of 33% when compared to the non-treated control (Table 11). Soybean treated with dicamba at 8.75 g ha^{-1} had more injury, lower plant height, and lower yield than those treated with 2.18 g ha^{-1} (Table 11).

For the second planting date (1 July), these two factors, soybean growth habit and dicamba rate, had a significant effect on visual injury 2 WAT, relative yield, and plant height 3 WAT (Table 8). The effect of soybean cultivar within growth habit was also significant (Table 8). Similar to the results from the 13 May planting date, visual injury and height reductions were greater in soybean of indeterminate growth than that of a determinate growth (Table 9). Differences were also observed in relative yield at the July planting date (Table 9). The three indeterminate soybean varieties had an average relative yield of 68%, whereas the two determinate varieties resulted in an average relative yield of 85%. Relative yield, averaged over dicamba rate, differed among the cultivars within each growth habit (Table 10). Of the soybean varieties of determinate growth, Bayer CZ 5445LL had less stunting and greater relative yield than Bayer CZ 5147LL (Table 10). Of the indeterminate varieties, Bayer CZ 4105LL had slightly higher injury and lower relative yield than HBK 4950LL and Armor 501LL, indicating that more research is needed to determine differences in cultivars among each growth habit.

Dicamba rate had a significant effect on all parameters measured (Table 11). Averaged across the determinate and indeterminate cultivars, dicamba at 2.18 g ha^{-1} caused less injury, plant height, and relative yield loss than the higher rate of 8.75 g ha^{-1}. Relative yields were reduced 12% following dicamba at 2.18 g ha^{-1} and 32% from the 8.75 g ha^{-1} rate (Table 11).

Regardless of dicamba rate, differences in visual injury were observed between indeterminate and determinate soybeans. Dicamba applied to soybean of indeterminate growth resulted in 28% injury 2WAT and reduced plant height 23 cm 3WAT. When applied to determinate soybean, 15% injury resulted and plant height was reduced 26 cm. These findings agree with previous work of Weidenhamer et al. (1989) who reported a greater negative effect on soybean of indeterminate growth exposed to dicamba at flowering than for determinate soybean that cease vegetative growth at the onset of flowering. Furthermore, Auch and Arnold (1978) state that in regard to soybean cultivars that are similarly susceptible to yield loss from dicamba, other differences in response have been observed, such as differences in height reductions. From this study, it can be concluded that indeterminate and determinate soybean cultivars can differ in response to low rates of dicamba; however, other factors may influence yield more significantly such as planting date, soybean cultivar, and rate of dicamba applied.

PRACTICAL APPLICATIONS AND CONCLUSIONS

Grain yield of HBK 4950LL and HALO 5.45LL soybean is highly sensitive to dicamba during the late vegetative/early reproductive growth stages, and observable injury is at best a moderate indicator of yield loss for both cultivars, as noted previously (Wax et al. 1969; Auch and Arnold 1978; Weidenhamer et al. 1989; Al-Khatib and Peterson 1999; Andersen et al. 2004; Kelley et al. 2005; Johnson et al. 2012; Griffin et al. 2013; Solomon and Bradley 2014). During these sensitive growth stages, depending upon the amount of dicamba exposure, injury is expressed 1 WAT and tends to increase until 3 to 4 WAT. Furthermore, damage to the soybean terminal, resulting in reduced plant height, appears to be a good indicator of yield reduction; however, other factors influence height reductions such as application timing and cultivar selection (Weidenhamer et al. 1989).

The plasticity of soybean makes it difficult to generalize the effects of dicamba (Auch and Arnold 1978). The plasticity of soybean can be expressed by examining these different cultivars. Greater recovery is expected in the mid-South from cultivars of late-maturing cultivars (maturity group 5) due to vegetative growth remaining for a longer period before flowering, allowing for more production of leaf area and nodes for pod formation. This longer period of vegetative growth allows for greater recovery of yield potential following herbicide-induced injury during early vegetative growth stages (Ritchie et al. 1994; Westgate 1999; Holshouser 2001); however, Wax et al. (1969) found that a determinate soybean may be more sensitive to exposure to dicamba during vegetative growth stages compared to an indeterminate soybean. Furthermore, yield loss resulting from dicamba injury may differ depending upon soybean growth habit; however, other factors may be of more influence.

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Table 1. Information for each trial conducted including: location, year, soybean cultivar and maturity group (MG), growth habit, planting date, and harvest date information for dicamba experiments.

Location	Year	Cultivar (MG)	Growth habit	Planting date	Harvest date
Marianna	2014	HBK 4950LL (MG IV)	Indeterminate	June 5	October 17
Marianna	2014	HALO 5.45LL (MG V)	Determinate	June 5	October 24
Marianna	2015	HBK 4950LL (MG IV)	Indeterminate	May 13	October 1
Marianna	2015	HALO 5.45LL (MG V)	Determinate	May 13	October 3
Marianna	2015	HBK 4950LL (MG IV)	Indeterminate	July 1	October 22
Rohwer	2014	HBK 4950LL (MG IV)	Indeterminate	June 18	October 23
Rohwer	2015	HBK 4950LL (MG IV)	Indeterminate	July 1	October 13
Rohwer	2015	HALO 5.45LL (MG V)	Determinate	June 6	October 13

Table 2. Planting date, harvest date, soybean cultivar, maturity group (MG), growth habit, and dicamba application timing at Marianna, AR in 2015.

Planting date	Harvest date	Cultivar (maturity group)	Growth habit	Application date
May 13	October 3	Bayer CZ 4105LL (MG 4.1)	Indeterminate	June 22
		HBK 4950LL (MG 4.9)	Indeterminate	July 7
		Armor 501LL(MG 5.0)	Indeterminate	July 9
		Bayer CZ 5147LL (MG 5.1)	Determinate	July 1
		Bayer CZ 5445LL (MG 5.4)	Determinate	July 7
July 1	October 22	Bayer CZ 4105LL (MG 4.1)	Indeterminate	August 4
		HBK 4950LL (MG 4.9)	Indeterminate	August 11
		Armor 501LL(MG 5.0)	Indeterminate	August 11
		Bayer CZ 5147LL (MG 5.1)	Determinate	August 11
		Bayer CZ 5445LL (MG 5.4)	Determinate	August 18

Table 3. The interaction effect of application timing and dicamba rate on observable injury to soybean and pod malformation. The main effect of application timing on observable injury, relative yield, and the average number of pods per plant for a HBK 4950LL soybean.[a] Data combined over years of studies conducted at Rohwer and Marianna, AR, in 2014 and 2015.

a

Application Timing.	Injury from dicamba (rates g ae ha^{-1})[b] 1 WAT[c] 2.18	1 WAT[c] 8.75	2 WAT 2.18	2 WAT 8.75	4 WAT Avg of rates	Plant height[d] Maturity Avg of rates	Relative yield[ef] Avg of rates	Pods Malformed 2.18	Pods Malformed 8.75	Pods No. $plant^{-1}$[e]
	%					cm	%	%		
V4	12 cde	24 a	24 d	37 a	26 c	83 c*	92 c*	20 e	23 de	54 a
V6	9 ef	12 cde	31 b	36 a	42 a	71 d*	91 c*	21 de	21 e	44 bc
R1	15 c	18 bc	26 cd	30 bc	43 a	66 d*	86 d*	28 cde	34 bc	49 b
R2	12 cde	15 c	19 e	24 d	38 b	72 d*	92 c*	26 cde	29 cde	49 b
R3	4 g	4 gh	20 e	7 g	18 d	89 bc	93 bc	29 cde	40 b	48 bc
R4	8 f	10 def	9 g	0	4 e	93 ab	94 abc	31 bcd	49 a	43 c
R5	4 gh	8 f	0	0	0	97 a	97 a	26 cde	24 de	46 bc
R6	1 gh	4 gh	0	0	0	99 a	96 ab	21 de	21 e	49 b

Means separated within paired columns using Fisher's protected LSD at $\alpha=0.05$.

[b] Injury rated on a scale from 0% to 100%, with 100 being plant death. Injury ratings of 0 were removed from statistical analysis.

[c] Abbreviation: WAT, weeks after treatment.

[d] Plant heights measured from the soil surface to the top of the central stem.

[e] (*) Denotes measurements significantly different than the nontreated check. The nontreated control measured 101 cm at maturity, yielded 3760 kg ha^{-1}, and averaged 55 pods $plant^{-1}$.

Table 4. The main effect of dicamba rate on observable injury to soybean, plant height, and relative grain yield for HBK 4950LL soybean.[ab] Data combined over years of studies conducted at Rohwer and Marianna, AR, in 2014 and 2015.

Rate	Injury 4 WAT[cd]	Relative yield[f]
g ae ha^{-1}	%	%
2.18	19 b	92 a*
8.75	23 a	94 b*

[a] Means separated within columns using Fisher's protected LSD at α=0.05.
[b] Data averaged over eight application timings (V4, V6, R1-R6).
[c] Abbreviation: WAT, weeks after treatment.
[d] Injury rated on a scale from 0% to 100%, with 100 being plant death.
[e] Plant heights measured from the soil surface to the top of the central stem.
[f] (*) Denotes measurements significantly different than the nontreated check. The nontreated control had a grain yield of 3750 kg ha^{-1}.

Table 5. The interaction effect of application timing and dicamba rate on observable injury to soybean and pod malformation. The main effect of application timing on observable injury to soybean, plant height, relative grain yield, and average number of pods per plant for HALO 5.45LL soybean.[a] Data combined over years of studies conducted at Rohwer and Marianna, AR, in 2014 and 2015.

Application Timing.	Injury from dicamba (rates g ae ha^{-1})[b] 1 WAT[c] 2.18	1 WAT 8.75	2 WAT Avg of rates	4 WAT 2.18	4 WAT 8.75	Plant height[d] Maturity[e] Avg of rates	Relative yield[e] Avg of rates	Pods Malformed 2.18	Pods Malformed 8.75	Pods No. $plant^{-1}$[e]
	%					cm	%	%		
V4	16 bc	25 a	44 a	20 f	30 e	69 c*	84 cd*	15 cdef	16 cdef	77 ab
V6	12 cde	19 b	36 b	42 bc	55 a	60 d*	84 cd*	13 ef	15 cdef	74 ab
R1	11 def	15 cd	27 c	38 cd	48 ab	63 d*	81 d*	19 cde	22 bcd	69 b*
R2	9 efgh	11 efg	20 d	29 e	34 de	73 c*	84 cd*	16 cdef	30 ab	65 b*
R3	4 j	8 efghi	14 e	18 f	18 f	81 b	99 a	12 f	14 ef	85 a
R4	6 ghij	11 def	10 e	8 g	9 g	90 a	90 bc	15 def	36 a	72 b
R5	5 hij	8 fghij	4 f	3 g	5 g	91 a	97 a	24 bc	17 cdef	75 ab
R6	5 hij	5 ij	3 f	2 g	6 g	89 a	95 ab	15 def	14 ef	77 ab

[a] Means separated within paired columns using Fisher's protected LSD at α=0.05.

[b] Injury rated on a scale from 0% to 100%, with 100 being plant death.

[c] Abbreviation: WAT, weeks after treatment.

[d] Plant heights measured from the ground to the top of the central stem.

[e] (*) Denotes measurements significantly different than the nontreated check. The nontreated control measured 89 cm at maturity, yielded 3700 kg ha^{-1}, and averaged 86 pods $plant^{-1}$.

Table 6. The main effect of dicamba rate on observable injury to soybean, plant height, relative grain yield, and number of pods per plant for HALO 5.45LL soybean.[ab] Data combined over years of studies conducted at Rohwer and Marianna, AR, in 2014 and 2015.

Rate	Injury[c] 2 WAT[d]		Plant height[e] Maturity[f]		Relative yield[f]		Pod No. plant^{-1}[f]	
g ae ha^{-1}	%		——cm——		%			
2.18	18	b	77	a	93	a*	81	a
8.75	22	a	73	b*	86	b*	67	b*

[a] Means separated within columns using Fisher's protected LSD at α=0.05.
[b] Data averaged over eight application timings (V4, V6, R1-R6).
[c] Injury rated on a scale from 0% to 100%, with 100 being plant death.
[d] Abbreviation: WAT, weeks after treatment.
[e] Plant height measured from the ground to the top of the central stem.
[f] (*) Denotes measurements significantly different than the nontreated check. The nontreated control measured 88 cm at maturity, yielded 2950 kg ha^{-1}, and averaged 86 pods plant^{-1}.

Table 7. Analysis of variance (α=0.05) of soybean injury, plant height, relative yield of indeterminate and determinate soybean.[a] Two studies were conducted at different locations at Marianna, Arkansas in 2015. Dicamba at 2.18 g ae ha^{-1} and 8.75 g ae ha^{-1} was applied at R1 growth stage to each soybean cultivar. Observable injury was rated 2 WAT[b] and heights were collected 3 WAT.

		May 13			July 1		
Factor	DF	Injury	Relative yield	Plant height	Injury	Relative yield	Plant height
Growth habit	1	**0.0482**	0.9100	**<0.0001**	**<0.0001**	**0.0003**	**<0.0001**
Dicamba rate	1	**<0.0001**	**<0.0001**	**<0.0001**	**0.0156**	**<0.0001**	**<0.0001**
Growth habit X Rate	1	0.4220	0.8004	0.1515	0.2298	0.9990	0.1123
Cultivar [growth habit]	3	**<0.0001**	**0.0126**	**<0.0001**	**0.0340**	**0.0003**	**<0.0001**
Rep & Random	3	0.0258	0.0442	0.7012	0.1024	0.5419	0.3792

[a] Soybean cultiavars evaluated included Bayer CZ 4105LL, HBK 4950LL, Armor 501LL, Bayer CZ 5147LL, Bayer CZ 5445LL.
[b] Abbreviation: WAT, weeks after treatment.

Table 8. The main effect of soybean growth habit on observable injury, relative yield, and plant height following an application of dicamba applied at R1 reproductive stage.[abc] Trials were conducted at Marianna, AR in 2015.

Planting date	Growth habit	Injury 2 WAT[de]		Relative yield[f]		Plant height 3 WAT[fg]	
		%				cm	
May 13	Indeterminate	42	a	79	a*	30	b*
	Determinate	38	b	79	a*	46	a*
July 1	Indeterminate	28	a	68	b*	25	b*
	Determinate	15	b	85	a*	38	a*

[a] Soybean cultivars evaluated including Bayer CZ 4105LL, HBK 4950LL, Armor 501LL, Bayer CZ 5147LL, Bayer CZ 5445LL.

[b] Data were averaged across dicamba rates (2.18 g ae ha^{-1} and 8.75 g ae ha^{-1}).

[c] Means separated within columns using Fisher's protected LSD at α=0.05.

[d] Injury rated on a scale from 0% to 100%, with 100 being plant death.

[e] Abbreviation: WAT, weeks after treatment.

[f] (*) Denotes measurements significantly different than the nontreated check. The nontreated check for indeterminate soybean planted May 13, 2015 yielded 3300 kg ha^{-1} with a height of 58 cm 3 WAT and the determinate soybean yielded 3820 kg ha^{-1} with a height of 71 cm. The nontreated check for the indeterminate soybean planted July 1, 2015 yielded 2590 kg ha^{-1} with a height of 48 cm 3 WAT and the determinate soybean yielded 2440 kg ha^{-1} with a height of 64 cm.

[g] Plant heights were measured from the ground to the top of the central stem.

Table 9. The main effect of soybean cultivar nested within growth habit on soybean observable injury, relative yield, and plant height following dicamba application at R1 reproductive stage.[ab] Trials were conducted at Marianna, AR in 2015.

Planting date	Growth habit	Soybean cultivar	Injury 2 WAT[d]		Relative Yield[e]		Plant height 3 WAT[ef]	
			———	%	———		cm	
May 13	Indeterminate	Bayer CZ[c] 4105LL	36	b	73	b*	33	c*
		HBK 4950LL	47	a	80	a*	28	d*
		Armor 501LL	44	a	85	a*	30	c*
	Determinate	Bayer CZ 5147LL	29	b	80	a*	41	b*
		Bayer CZ 5445LL	49	a	79	ab*	51	a*
July 1	Indeterminate	Bayer CZ 4105LL	33	a	57	c*	28	c*
		HBK 4950LL	25	b	74	b*	23	d*
		Armor 501LL	26	b	75	b*	25	c*
	Determinate	Bayer CZ 5147LL	16	c	73	b*	33	b*
		Bayer CZ 5445LL	14	c	98	a	49	a*

[a] Data were averaged across dicamba rates (2.18 g ae ha^{-1} and 8.75 g ae ha^{-1}).
[b] Means separated within columns using Fisher's protected LSD at α=0.05.
[c] Injury rated on a scale from 0% to 100%, with 100 being plant death.
[d] Abbreviation: WAT, weeks after treatment.
[e] (*) Denotes measurements significantly different than the nontreated check. For the May 13, the nontreated check for Bayer CZ 4105LL soybean yielded 3440 kg ha^{-1} with a height of 58 cm, HBK 4950LL soybean yielded 3330 kg ha^{-1} with a height of 60 cm, Armor 501LL soybean yielded 3150 kg ha^{-1} with a height of 53 cm, Bayer CZ 5147LL soybean yielded 3510 kg ha^{-1} with a height of 64 cm, and Bayer CZ 5445LL soybean yielded 4120 kg ha^{-1} with a height of 76 cm. For the July 1, the nontreated check for Bayer CZ 4105LL soybean yielded 2220 kg ha^{-1} with a height of 51 cm, HBK 4950LL soybean yielded 2790 kg ha^{-1} with a height of 52 cm, Armor 501LL soybean yielded 2770 kg ha^{-1} with a height of 43 cm, Bayer CZ 5147LL soybean yielded 2840 kg ha^{-1} with a height of 58 cm, and Bayer CZ 5445LL soybean yielded 2050 kg ha^{-1} with a height of 67 cm.
[f] Plant heights were measured from the ground to the top of the central stem.

Table 10. The main effect of dicamba rate on soybean observable injury, relative yield, and plant height following dicamba application at R1 reproductive stage.[abc] Trials were conducted at Marianna, AR in 2015.

Planting date	Rate	Injury 2 WAT[ed]		Relative yield[f]		Plant height 3 WAT[fg]	
	g ae ha^{-1}	———	%	———		cm	
May 13	2.18	34	b	84	a*	43	a*
	8.75	46	a	74	b*	33	b*
July 1	2.18	19	b	88	a*	38	a*
	8.75	23	a	68	b*	28	b*

[a] Soybean cultivars evaluated including Bayer CZ 4105LL, HBK 4950LL, Armor 501LL, Bayer CZ 5147LL, Bayer CZ 5445LL.

[b] Data were averaged across growth habit (indeterminate, determinate).

[c] Means separated within columns using Fisher's protected LSD at α=0.05.

[d] Injury rated on a scale from 0-100, with 100 being plant death.

[e] Abbreviation: WAT, weeks after treatment.

[f] (*) Denotes measurements significantly different than the nontreated check. Averaged across cultivars, the nontreated check for soybean planted May 13, 2015 yielded 3509 kg ha^{-1} with a height of 64 cm 3 WAT. The nontreated check for soybean planted July 1, 2015 yielded 2531 kg ha^{-1} with a height of 53 cm 3 WAT.

[g] Plant heights were measured from the ground to the top of the central stem.

CHAPTER 3: DICAMBA EFFECTS ON SOYBEAN POD AND SEED: POD MALFORMATION AND OFFSPRING RESPONSE

ABSTRACT

As the adoption of the Roundup Ready® Xtend technology continues and the use of dicamba increases across the United States, the risk of injury to sensitive crops is of concern. There is a clear understanding that dicamba injury can have detrimental effects on soybean growth and yield; however, research is needed to evaluate the effects of dicamba on soybean pods and seeds. Field experiments were conducted in 2014 and 2015 to determine the effects of dicamba on soybean growth and yield. In the following two years, offspring from these studies were evaluated in the greenhouse. Similar experiments were conducted in field trials at the Lon Mann Cotton Research Station in Marianna, Arkansas. The objectives of these studies were to determine 1) if seedling response to dicamba applied to parent plants the previous year was dependent upon application timing and/or dicamba rate and 2) if pods in a specific location on the plant were affected more than others based on growth stage when dicamba was applied. Soybean offspring were negatively affected by late-season exposure to low dicamba rates. Seed germination and offspring emergence were negatively affected as a result of dicamba applications, particularly following mid to late season applications. Of the emerged seedlings collected from the HBK 4950LL soybean, 91% of the offspring treated at R6 with 8.75 g ae ha^{-1} expressed injury in a controlled environment. Under field conditions, offspring from these same plants treated at R6 resulted in yield reduction of 20% when compared to the nontreated. When parent plants were treated prior to R3, injury to offspring was reduced.

The amount of pod malformation differed depending on the application timing; however, no relationship existed between pod malformation and offspring response for soybean injury, vigor, and emergence. The greatest percentage of pod malformation resulted from dicamba applied at full pod (R4), with 43% malformation. In an additional experiment, seeds collected from the bottom portion of plants appeared to be affected more by dicamba than seeds collected from the top of plants. Averaged across application timings and dicamba rate, only 70% of seeds collected from the bottom of the plant emerged compared to the nontreated. Offspring from seed collected from the bottom of plants was injured 22% and had an average plant height of 14 cm at V2, a 7% reduction in height when compared to the nontreated. Conversely, 79 to 83% of the seeds collected from the top and middle of plants emerged and expressed visual dicamba injury of 15 to 16% with height reductions of 5 to 6%. On average, malformed pods were distributed equally between the top, middle, and bottom of plants.

Nomenclature: Dicamba; soybean, *Glycine max* (L.) Merr.

Key words: dicamba, drift, tank contamination, progeny, crop injury

INTRODUCTION

With the recent approval of the Roundup Ready® Xtend™ technology (Monsanto Company, St. Louis, MO 63167), growers will have an additional weed management option in soybean and cotton (*Gossypium hirsutum* L). The new technology will allow for over-the-top application of glyphosate and dicamba in soybean and glyphosate, dicamba, and glufosinate in cotton. This technology will provide an additional weed control option for Palmer amaranth (*Amaranthus palmeri (S.) Wats.*); however, off-target movement of dicamba is of concern, especially to soybean without the resistant trait. Dicamba at any rate can be injurious to susceptible soybean cultivars, resulting in significant yield loss at particular growth stages (Barber et al. 2015). Dicamba at 35 g ha^{-1} severely reduced soybean yield when applied during flowering (Wax et al. 1969). Averaged across rate (2.18 g ha^{-1} and 8.75 g ha^{-1}), dicamba applied at flowering caused 43% injury to soybean 4 weeks after treatment (WAT) (Barber et al. 2015). When applied at R5 or R6, injury was insignificant and yield was not reduced. Hence, severity of visible injury symptoms and yield reduction decreases as dicamba is applied to soybean later in the reproductive stages; however, other concerns may arise. For instance, when soybean is exposed to dicamba during reproductive growth stages, the herbicide may be stored in the seeds of the soybean plant (Thompson and Egli 1973).

Reduction in seed number and seed viability occurs when weeds are treated with dicamba at or near flowering (Biniak and Aldrich 1986; Fawcett and Slife 1978; Jha and Norsworthy 2012; Maun and Cavers 1969). The movement of these herbicides in the plant is similar to the transport of photosynthates. When photosynthates are stored in the seed, herbicides may be stored in the seed as well (Thompson and Egli 1973). As seen in weedy species, herbicides stored in soybean seed can decrease germination and can be injurious to developing seedlings

(Wax et al. 1969). Glyphosate applied as a harvest aid to soybean during the late reproductive growth stages (R5, R6, R7) reduced seed germination and percentage of normal seedlings of soybean (Ratnayake and Shaw 1992). Glyphosate at 560 g ha^{-1} applied at R5 resulted in seed germination of 66% and reduced normal seedling percentage to 45%. Conversely, sub-lethal rates of dicamba applied during later reproductive growth stages reduced soybean seed germination and emergence (Thompson and Egli 1973). No seed from plants treated with dicamba at 30 g ha^{-1} or 220 g ha^{-1} at pod filling (R5) was classified as normal in a standard germination test and at least 70% of the seeds were classified as "dead" with the remaining exhibiting abnormal germination. Emerged seedlings expressed malformed first trifoliate leaves and a reduction in biomass was observed compared to the nontreated. Thompson and Egli (1973) also reported that differences in offspring vigor and emergence were observed depending on where the seed was harvested on the parent plant. Of the seeds harvested, only 50% of the seeds harvested from the top and 36% of the seed from the bottom of plants germinated normally when treated with dicamba at 30 g ha^{-1} at flowering. Of the emerged seedlings, those from the top of plants treated at flowering and the top and bottom of plants treated at pod filling were less vigorous than offspring of non-treated plants. Hence, application timing and dicamba rate may interact to negatively affect soybean offspring.

The beginning of reproductive growth is identified by first flower. Soon after first flower, or R1, soybean begins to produce pods at R3, or when a pod of 0.5 cm is found on one of the upper four nodes. During this time, partial compensation from temporary stress can occur in soybean, but as the plant matures this ability to compensate will decrease (Doss et al. 1973). However, the long flowering period of soybean is one reason these plants can compensate so well. At R4 growth stage, or full pod, soybean shows rapid pod growth and the beginning of seed development. This stage is the most crucial period for seed yield and any stress from R4-R6 causes more yield reduction than at any other time in the growing season (Doss et al. 1973). Toward the end of the R4 growth stage, nutrient accumulation in the leaves peaks and then begins the process of redistributing to the seed. Seed accumulation will continue until shortly after R6.5 with about 80% of total seed dry weight accomplished. At R6, or full seed, total pod weight peaks and growth rate of the grain is rapid but will slow at R6.5 and cease by R7. Soybean then begins to obtain a mature color and reach full maturity at R8 (Doss et al. 1973).

The objective of this experiment were to determine if seedling response to dicamba applied to parent plants the previous year was dependent upon application timing and/or dicamba rate and 2) if pods in a specific location on the plant were more affected more than others based on growth stage when dicamba was applied.

MATERIALS AND METHODS

Field Trials. In 2014 and 2015, field trials were conducted to determine the response of soybean to dicamba applied at vegetative and reproductive growth stages. Trials were conducted at two locations, the Lon Mann Cotton Research Station in Marianna, Arkansas on a Calhoun silt loam soil and the Rohwer Research Station near Rohwer, Arkansas on a Sharkey clay soil. Studies were conducted on two dicamba-susceptible cultivars; HBK 4950LL, an indeterminate (MG IV) cultivar and HALO 5.45LL, a determinate soybean cultivar (MG V). For each study, two low rates of dicamba were evaluated: 1/64X (8.75 g ha^{-1}) and 1/256X (2.18 g ha^{-1}) of a normal 1X rate (560 g ha^{-1}). Applications were made at the V4 and V6 stages and at each reproductive stage starting with R1 and ending with R6. Experiments also included a nontreated check at all locations for comparison purposes. Fertility, weed control, irrigation, and overall management practices implemented were research-based University of Arkansas Extension recommendations (Arkansas Soybean Production Hankbook-MP197).

Offspring Evaluations. All greenhouse studies were conducted at the University of Arkansas Altheimer Laboratory in Fayetteville, Arkansas. Seeds collected from the 2014 field trials were evaluated in 2015 and seeds collected from the 2015 field trials were evaluated in 2016.

Application Timing and Dicamba Rate. Seed samples collected from plots containing the same treatment in the field were combined. Fifteen seed from each sample were planted in the greenhouse to evaluate the effect of the dicamba rate and application timing on the developing offspring. Seed from the indeterminate and determinate cultivar were evaluated in separate experiments. Seed were planted in potting soil in 30- by 15- by 10-cm-deep trays with 15 seeds per tray, and each treatment was replicated four times. The trays were arranged on the greenhouse bench in a randomized complete block design and were watered as needed. Day/night temperatures were set to 32/22 C with a 16-hour photoperiod. Injury and plant vigor resulting from the field application of dicamba were visually evaluated at the second trifoliate and average heights were determined. Average soybean heights were collected by measuring three random soybean plants per tray from the soil surface to the top of the central stem or terminal. Visible estimates of injury were evaluated using a scale of 0 to 100%, where 0 equals no injury and 100 equals complete plant death. Soybean vigor was evaluated using a scale of 1 to 5, where 1= extremely low vigor (delayed and/or reduced emergence; likely extremely poor emergence under field conditions), 2= poor vigor (slow initial growth and 30 to 60% reduction in emergence in the field), 3=moderately low vigor (slight reduction in emergence likely under good field conditions), 4= moderately high vigor (slight reduction in emergence in fields having suboptimal conditions), 5= extremely high vigor (seedlings quickly emerge; exhibit rapid growth; likely to emerge under a wide array of field conditions). Although a standardized definition of vigor satisfactory to most investigators has yet to be realized, the concept of vigor and its importance in crop development are well accepted (Pollock and Roos 2012). Stand counts were collected on the number of plants emerged and the number of plants showing malformations. Aboveground biomass was harvested from soil level and fresh weight was measured at the second trifoliate stage.

In 2014 and 2015, a portion of the seed sample from each application timing treated with dicamba at 8.75 g ha^{-1} was sent to the Arkansas State Plant Board laboratory for laboratory testing. As a result of observing a significant decrease in germination during the later reproductive growth stages, in 2014, accelerated aging (AA) tests were also performed on the seed samples collected from soybean treated at R3, R4, R5, and R6. Accelerated aging uses individual or combinations of simulated environmental variables to speed up the normal aging process of seed. These variables are high or low temperatures, high relative humidity, oxygen, sunlight, and vibration. Results from AA tests are helpful in determining long-term effects of expected levels of stress on seed within a short timeframe (Long et al. 2008). Under normal conditions, expected AA results should be within 20 percentage points of normal germination results. The AA test used for this study exposed soybean seed to high temperature and high relative humidity over a short period of time. Seed samples were tested at the Arkansas State Plant Board using the following procedure: 38 g of soybean seed were evenly distributed onto a crisper, or wire mesh, and placed in an acrylic chamber at 41 C. A small dish, approximately 30- by 15-cm containing 40 mL of distilled water was placed underneath crisper at the bottom of the chamber. After 72 hours, seed were removed and a standard germination test was conducted to evaluate seed vigor.

In 2015, seed collected from each of the two 2014 studies in Marianna, AR were planted in a field trial in Marianna, AR to determine if results similar to those of the greenhouse were observed. The experiment was arranged as a randomized complete block design, using the same field plan from the trial of where the seed were harvested. Each plot consisted of two 7.6 m long rows spaced 97 cm apart and 220 seeds were planted per row. Similar to the greenhouse study, visual injury ratings, plant vigor, and stand counts were collected at the second trifoliate stage, or V2. Percentage of emerged plants was calculated by dividing the number of emerged plants by the number of seed planted in the row. Also, the number of plants emerged and the number of injured plants were documented for a meter of row to calculate a percentage of injured plants. At crop maturity, soybean plots were harvested using a small-plot combine, and seed yields were adjusted to 13% moisture content. Relative yields were calculated to determine the percentage of yield loss in each plot compared to the nontreated check.

Field trials and greenhouse studies were analyzed separately, and experiments were analyzed separately based on soybean cultivars. Each experiment was designed as a factorial arrangement of treatments in a randomized complete block design with two factors, eight application timings and two dicamba rates. Data for visual injury, plant vigor, plant height, percentage emergence, percentage injured plant, and soybean offspring yield were subjected to analysis of variance (ANOVA) using JMP 11.0.0 (SAS® Institute Inc., Cary, NC 27513) to test for the significant effects of dicamba rate, treatment timing, and the interaction of both factors. Dicamba rates and application timings were considered fixed effects in all models. For each study, environment and replications were considered random effects. Treatment means were separated using Fisher's protected LSD at α=0.05. Although, means from nontreated plots were not included in the ANOVA, Tukeys HSD (Honest Significant Difference) test was used to find out which specific means are different from the nontreated and were denoted with an asterisk.

Pod Location. In addition to evaluating the factors of application timing and dicamba rate, it was of interest to determine if pod location on the soybean plant influenced offspring. In 2014, prior to combine harvest, ten random plants were collected in a meter of row from each plot. Plant samples from each cultivar (indeterminate and determinate) were collected. Plant samples were stored in a temperature-controlled environment at 23 C for approximately one month. Using plant samples collected from the meter row, each plant was divided into thirds. Plants were cut into top, middle, and bottom parts based on the number of nodes on the plant. A node is the part of the stem where a leaf is (or has been) attached. Data were gathered on the number of pods and the number of malformed pods in each third of the plant. Pods and seed were collected to later assess the effect of dicamba on the subsequent generation (offspring) through greenhouse trials. Once collected, seeds were stored in a temperature-controlled environment at 5 C. As each plant was sectioned in the laboratory, pods were counted, including the number of malformed pods in each plant section. Seed was harvested from pods and seed containing the same treatment/pod positon were combined. Fifteen seed were randomly chosen from each section and planted in individual trays filled with potting soil. Seed were planted in potting soil in 30- by 15- by 10-cm-deep tray and each treatment was replicated four times. The trays were arranged on the greenhouse bench in a completely random design and were watered as needed. Day/night temperatures were set to 32/22 C with a 16-hour photoperiod. Offspring plants were evaluated as in the previous greenhouse study. According to ANOVA, no significant effect of cultivar was observed on any of the parameters evaluated and resulted in data being combined across the indeterminate and determinate cultivars.

This study was analyzed as a split-plot design, with the whole plot containing two factors (application timing and dicamba rate) and each subplot split into pod position (top, middle, bottom) based on where the seeds were collected. Data for visual estimates of emergence, injury, vigor, plant height, fresh weight, and percentage of malformed pods within each section were subjected to ANOVA using JMP 11.0.0 (SAS® Institute Inc., Cary, NC 27513) to test for the significant effects of application timing, dicamba rate, and pod position. Application timing, dicamba rate, and pod position were considered fixed effects in the model, whereas soybean cultivar, environment, replications and subsamples were considered random effects. Treatment means were separated using Fisher's protected LSD at $\alpha=0.05$.

RESULTS AND DISCUSSION

HBK 4950LL Offspring. Soybean seed harvested from parent plants treated with a low rate of dicamba had decreased germination when the application occurred during the latter reproductive growth stages, beginning with treatments applied at pod fill. Similar to the findings of Ratnayake and Shaw (1992) on the effects of glyphosate, soybean germination through standard and accelerated aging tests was greatly reduced, to as low as 20% for standard and 5% for accelerated aged seed of the untreated plants, from parent plants treated with dicamba from R4 through R6 (Figure 1). The lowest germination occurred for seeds treated with dicamba at 8.75 g ha^{-1} at R5; however, neither of the factors evaluated had a significant effect on emergence results (data not shown). Even though seeds expressed poor germination, emergence did occur in the greenhouse; albeit, vigor of offspring was greatly reduced when parent plants were exposed to low rates of dicamba after pod fill, similar to results in previous literature (Thompson and Egli 1973). Reduced plant vigor was observed not only in emergence, but also in plant height and the percentage of injured plants (Table 1).

Visible injury in the form of dicamba-like symptoms was not apparent until plants reached the first trifoliate stage. At this time, the percentage plants showing symptomology increased significantly when dicamba was applied to parent plants at the R4, R5, or R6 growth stage, especially with the higher dicamba rate of 8.75 g ha^{-1}. Dicamba applications made prior to R3 caused insignificant effects on offspring (<10% injury). Dicamba at 8.75 g ha^{-1} applied to parent plants at the R4 and R5 stages resulted in 58 and 64% of the emerged plants expressing dicamba-like injury, respectively (Table 1). When applied at the R6 stage, the percentage of emerged plants expressing injury was 91%. The lower rate of 2.18 g ha^{-1} reduced the number of offspring expressing injury from 91 to 50%. Overall, visual injury observed on soybean offspring depicted a similar trend, increasing up to 40% from later dicamba applications (Table 1). Visible injury in offspring from application at the R5 and R6 stages was 21% and 23%, respectively, when dicamba was applied at 2.18 g ha^{-1} (Table 1).

Injury from soybean offspring resulting from dicamba exposure was expressed as reduction in plant height and fresh weight. The most severe stunting was following dicamba at 8.75 g ha^{-1} applied at the R6 stage to parent plants, with a 35% reduction in height of offspring (Table 1). When the same rate was applied at the R4 and R5 stages to parent plants, offspring expressed 17% and 13% reduction in plant height. The R4 and R5 applications resulted in a 20% reduction in fresh weight, whereas only a 15% reduction resulted from the R6 treatment. This could be explained by the increase in offspring emergence. Dicamba rate had much less effect on plant height reductions compared to visual symptoms; however, plant height was reduced 25% as a result of the higher dicamba rate applied at the R6 stage (Table 1).

In the field, similar observations were collected on soybean offspring from parent plants treated with dicamba with a larger number of plants expressing injury compared to untreated plants. Also an interaction between application timing and dicamba rate was observed on offspring emergence (p=.0305) (data not shown). Emergence as low as 61% was observed as a result of dicamba at 8.75 g ha^{-1} applied at the R5 stage; emergence was 84% when treated with dicamba at 2.18 g ha^{-1} (Table 2). Similar results were observed in offspring from parent plants treated at R4. Unlike results from the greenhouse, there was no interaction between application timing and dicamba rate on the percentage of injured plants (p=.2560) (data not shown). Averaged across rates, the percentage of offspring plants expressing injury ranged from 45 to 67% when treated at or prior to the R3 stage and increased up to 96% following applications made at R5 (Table 2). Injury of offspring from parents treated with dicamba at 8.75 g ha^{-1} at R4, R5, or R6 was 75 to 83% (Table 2). Minimal injury was observed in offspring from plants treated during vegetative growth.

Dicamba symptomology observed on offspring plants had a marginal influence on the relative grain yield of the same plants. Relative grain yield of the offspring from the HBK 4950LL soybean ranged from 86 to 100% of the nontreated (Table 2). The greatest yield loss was from offspring collected from parent plants treated with 8.75 g ha^{-1} of dicamba at R6, but was not statistically different than the nontreated control. It is difficult to determine the cause of yield loss in field plots because reduced offspring emergence or severe injury did not always result in yield reductions. (Table 2). Minimal to no differences were observed in offspring yield between the two rates at each growth stage.

HALO 5.45 Offspring. In the laboratory test, seeds collected from the determinate cultivar were less negatively affected than the indeterminate, HBK 4950LL, cultivar by dicamba, especially for treatments applied at R6. Standard germination ranged from 41 to 79%, whereas germination in the accelerated aging test ranged from 7 to 21% when soybean was treated once with dicamba at 8.75 g ha^{-1} from R4 through R6 (Figure 2). The lowest germination followed the R5 treatment of the parent plants (Figure 2). According to these lab results of treatments applied at R3, R4, and R5, differences in offspring emergence would be expected based on when dicamba exposure occurs. However, in the greenhouse, emergence was similar among treatments applied at R3 through R5, ranging from 86 to 92% when compared to the nontreated check (Table 3).

As observed with the HBK 4950LL cultivar, the percentage of injured HALO 5.45LL plants increased as dicamba was applied later in the growing season, and dicamba had minimal effects on offspring when parent plants were treated prior to R3 (Table 3). Dicamba at 8.75 g ha^{-1} applied at R4 and R5 to parent plants resulted in 68 and 76% of the offspring expressing injury, with overall injury ratings increasing to 43% (Table 3). A similar response was observed from R6 applications. As seen in previous research, injury observed in soybean offspring was reduced as dicamba rate is lowered (Thompson and Egli 1973). Following the lower rate of dicamba at 2.18 g ha^{-1} applied at R4 and R5 to parent plants, the percentage of injured offspring was 44 and 45%, with an overall injury rating of 21% (Table 3). Similarly, the lower dicamba rate applied at R6 reduced the percentage of injured plants from 84 to 61% and the overall injury rating from 47 to 29% (Table 3).

Plant vigor ratings were collected at the same time as the injury ratings and followed a similar trend. Reductions in plant vigor were observed in seeds collected from soybean treated at or after the beginning pod stage (R3) and differences in plant vigor were distinguishable between the two rates. Although plant vigor was rated at V2, low plant vigor was observed soon after soybean emergence and appeared worse in plots with offspring from plants treated at later growth stages.

Dicamba effects on soybean offspring were also expressed in plant height reductions and fresh weight; however, dicamba rate had no interactive effect with growth stage on fresh weight (p=.2309). Dicamba at 8.75 g ha^{-1} applied at R6 resulted in a 48% reduction in plant height (Table 3). When applied at R4 and R5, plant height was reduced 37% and 33% (Table 3). Averaged across dicamba rate, reductions in fresh weights, ranged from 22 to 31% compared to nontreated plants (Table 3). Fresh weight was influenced not only by reduced emergence, but also reduced plant vigor.

For dicamba at 8.75 g ha^{-1}, emergence results from field trials were more similar to lab results than to greenhouse results. Offspring of plants treated with dicamba at 8.75 g ha^{-1} applied at R4 and R5 resulted in 76% and 57% emergence, where as 100% emerged from the parent plants treated at the R6 stage of growth (Table 4). After emergence, the number of seedlings expressing injury in the field increased up to 84% as dicamba was applied later in the growing season; however, no interaction was observed between dicamba rate and application timing. Plots containing offspring from parent plants treated with dicamba at 8.75 g ha^{-1} at R5 resulted in the largest percentage of injured plants and expressed an overall injury rating of 88% (Table 4). Significantly less injury was observed in offspring when this rate was applied at R4 and R6 (Table 4). The lower dicamba rate of 2.18 g ha^{-1} applied at R5 reduced percentage of injured plants 13% and visual injury from 88 to 59%, leading to the conclusion that dicamba rate does influence offspring response of the HALO 5.45LL soybean (Table 4). Plant vigor followed a similar trend in the field as observed in the greenhouse, with a decrease in offspring as dicamba was applied later in the growing season (Table 4).

Neither dicamba rate nor application timing had a significant effect on soybean offspring grain yield (data not shown); however, similar to the HBK 4950 offspring, slight differences were observed in relative grain yield. Other than 8.75 g ha^{-1} dicamba applied at R6, relative grain yields did not differ between treatments. (Table 4).

Soybean Pod Malformation. Data collected from each cultivar (determinate and indeterminate) on pod formation were combined. Pod malformation was affected by an interaction of application timing and dicamba rate. The number of malformed pods increased up to 43% as dicamba was applied to parent plants at later growth stages, particularly when applied at the R4 growth stage (Table 5). The number of malformed pods collected from plants treated at R4 was reduced by nearly 50% when the rate was decreased from 8.75 g ha^{-1} to 2.18 g ha^{-1}. Other than when applied at R4, no significant differences were observed between dicamba rates on the percentage of pod malformation observed within each application timing. Dicamba at 8.75 g ha^{-1} applied at the R1 to R3 stages resulted in 30% of the total pods expressing malformation (Table 5). When applied during vegetative development (V4, V6) or a late reproductive growth stage (R6), 19 to 21% of pods were malformed. As seen in table 5, injury ratings from this experiment were similar to the previous experiments with up to 57% injury following dicamba at 8.75 g ha^{-1} applied at the R5 stage with similar injury following R6 application. In addition, it was determined that pod malformation was not correlated with damage to offspring ($p=0.218$).

Pod position had an effect on the response of offspring from soybean plants exposed to dicamba (Table 6). Negative effects were observed in the form of reduced emergence and lowered seedling vigor. Seed collected from the bottom of the plant expressed greater visual dicamba symptoms and lower emergence than seeds collected from the top of the plant (Table 6). On average, 6 out of 15 seeds collected from the bottom of the plant emerged, whereas 9 out of 15 seeds collected from the top of the plant emerged. Furthermore, offspring from the bottom of the plant expressed reduced plant vigor and reduced plant height, resulting in a reduction in fresh weight. Fresh weight was reduced 35% in offspring from the bottom of plant, whereas 24 to 31% reduction was observed in offspring collected from the top and middle of the plant (Table 6). Seedlings from the bottom portion of the plant reduced plant height 7%, and a 5% reduction was observed in seedlings from the top of the plant. Although, nontreated seeds from the bottom of the plant were much less vigorous than those collected from the other two pod locations, the effects of dicamba between pod locations were distinguishable in the severity of dicamba injury and plant height reductions.

PRACTICAL APPLICATIONS AND CONCLUSIONS

From these studies, it is concluded that carryover of dicamba residues into soybean offspring is likely when parent plants are exposed to low rates of the herbicide, particularly when exposure occurs during or after R3. When the parent plant is exposed to dicamba after the R3 stage, foliar symptoms may not be apparent; however, dicamba symptomology was observed in offspring of plants treated at R3 with as little as 2.18 g ha^{-1} dicamba (1/256X rate for over-the-top use in dicamba-resistant soybean). It is assumed that dicamba in the plant is no longer being transported to the soybean leaves, but rather is moving to the sink of the plant, which includes the pods and developing seeds. Negative effects were seen in seed germination, seedling emergence, plant height, and seedling vigor as well as an increase in pod malformation. Germination percentage of offspring through standard and accelerated aging tests was greatly reduced from parent plants that were exposed to low rates of dicamba at the R4 through R6- growth stages. When soybean seed were planted in the greenhouse and field, germination and vigor were greatly reduced at these stages as was expected based on data from laboratory tests. The emerged seedlings of plants treated from R4 through R6, showed a high percentage of leaf malformation at V2 and damage to the plants caused by the low rates of dicamba. The rate of dicamba during the exposure plays a significant role in determining the likely amount of injury to the offspring. When the dicamba rate applied to parent plants was reduced from 8.75 to 2.18 g ha^{-1}, overall injury to subsequent offspring decreased significantly from 40% to 23%. Based on these results, it is likely that a single exposure to a dicamba rate lower than 2.18 g ha^{-1} will result in even less injury to offspring, but symptoms could arise.

These results make clear the effect of off-target dicamba injury to non-dicamba-resistant soybean in reproductive stages. It is important to note that regardless of the rate applied to parent plants, no dicamba residue could be identified in the seed 14 days after treatment when samples were submitted to multiple labs for analysis (data not shown). This is just another indication that soybean appears to have more sensitivity to dicamba than current analytical techniques for quantifying the presence of dicamba in soybean tissues. This research continues to build on earlier research showing that low rates of dicamba can cause delayed maturity, reduced seed quality, and the occurrence of pod malformation and malformed offspring (Thompson and Egli 1973).

Due to application restrictions, non-traited soybean exposure to dicamba is more likely to occur earlier in the year during vegetative growth than later in the year. However, with a wide range of planting dates in the mid-South US, often over a 3-month period, and herbicide applications that allow dicamba to be applied through the R1 stage to dicamba-resistant soybean, exposure of non-resistant soybean plants to dicamba during later growth stages is plausible. The implication that dicamba can be transported to the seed of susceptible cultivars could have significant impacts on producers who grow seed of conventional or other transgenic lines that are not resistant to the dicamba herbicide. As the adoption of dicamba technology increases, it will be important for growers to follow a stringent application program to decrease the potential of off-target movement of this herbicide. In addition, it appears that utilizing dicamba formulations that are less volatile will be necessary to further reduce potential for off-target movement.

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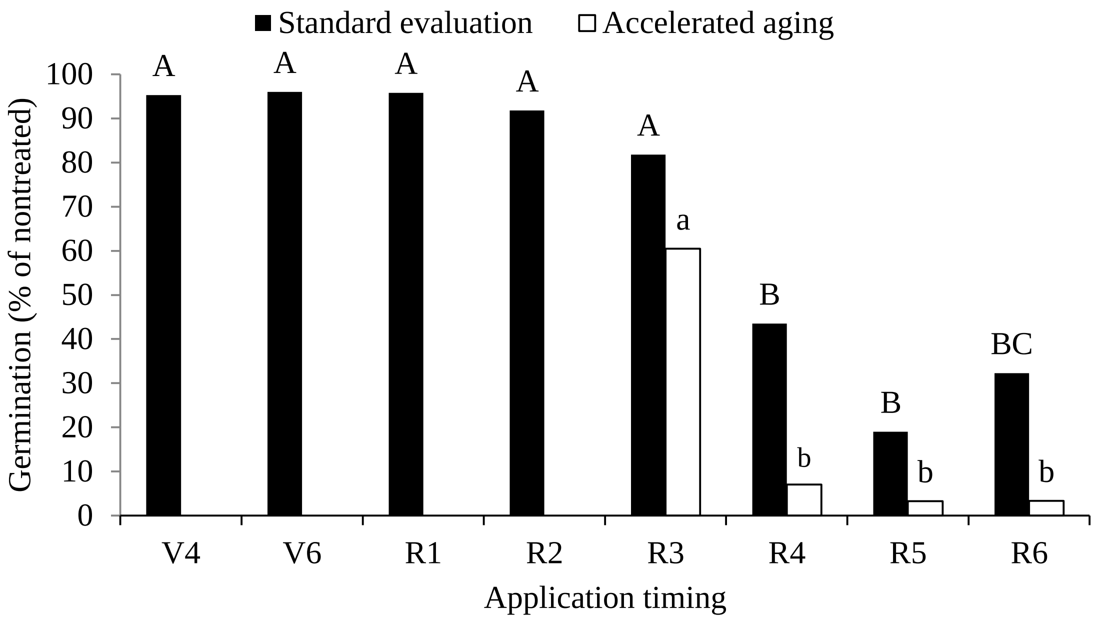

Figure 1. Soybean germination based on a standard evaluation and accelerated aging test for seed collected from HBK 4950LL cultivar that were treated with dicamba at 8.75 g ae ha^{-1} in 2014 and 2015 at Marianna, AR. Standard evaluation results were averaged over 2014 and 2015 (LSD=22). Accelerated aging results were from 2015 only and were not conducted on seed treated prior to R3 (LSD=22). Treatment means were separated using upper case letters for standard evaluation and lower case letters for accelerated aging.

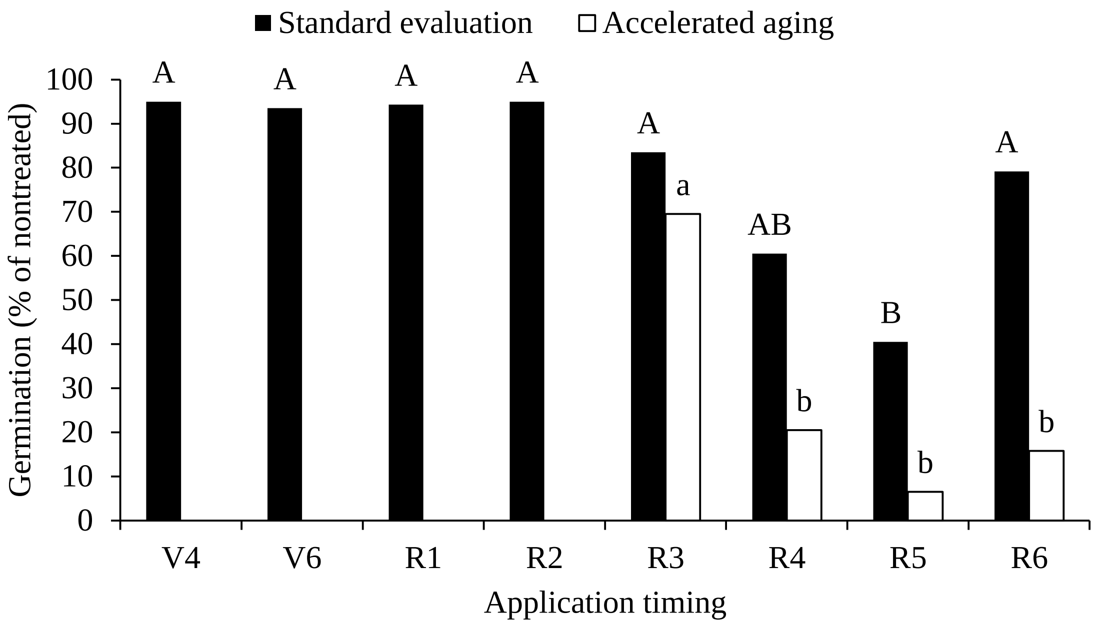

Figure 2. Soybean germination based on a standard evaluation and accelerated aging test for seed collected from HALO 5.45LL cultivar that were treated with dicamba at 8.75 g ae ha^{-1} in 2014 and 2015 at Marianna, AR. Standard evaluation results were averaged over 2014 and 2015 (LSD=26). Accelerated aging results were from 2015 only and were not conducted on seed treated prior to R3 (LSD=20). Treatment means are separated using upper case letters for standard evaluation and lower case letters for accelerated aging.

Table 1. The interaction effect of application timing and dicamba rate on soybean offspring emergence, percentage of injured plants, observable injury, vigor, plant height, and fresh weight of aboveground biomass.[a] Offspring seed was collected from HBK 4950LL soybean cultivar. In 2014 and 2015, parent plants were treated with dicamba at Marianna, AR. Offspring plants were evaluated in a greenhouse at Fayetteville, AR in 2015 and 2016. Data were collected from offspring at the second trifoliate stage.

Parent app. timing	Rate	Injured plants[b]		Injury[c]		Vigor[d]		Plant height[ef]		Fresh weight[f]	
	g ae ha^{-1}	——— % ———						cm		g $tray^{-1}$	
V4	2.18	0	d	1	f	5	ab	23	a	19	abcd
	8.75	0	d	2	ef	5	a	23	a	20	a
V6	2.18	0	d	3	ef	5	ab	23	a	19	abcd
	8.75	5	cd	4	ef	5	ab	22	a	19	abc
R1	2.18	0	d	2	ef	5	ab	22	a	19	abc
	8.75	2	d	4	ef	4	bc	24	a	18	abcde
R2	2.18	0	d	4	ef	4	bc	24	a	20	a
	8.75	8	cd	6	ef	4	bc	24	a	19	abc
R3	2.18	4	cd	3	ef	5	ab	23	a	19	abc
	8.75	22	c	9	de	4	bc	21	ab	19	abcd
R4	2.18	54	b	17	cd	4	cd	21	ab	19	abcd
	8.75	58	b	25	b	3	e	19	bc*	16	e*
R5	2.18	59	b	21	bc	3	e	21	ab	19	abc
	8.75	64	b	38	a	2	f	20	b	16	de*
R6	2.18	50	b	23	bc	3	e	20	b	20	a
	8.75	91	a	40	a	2	f	15	c*	17	cd*

[a] Means in a column with the same letter do not differ according to Fisher's protected LSD at α=0.05.
[b] Percent calculated from the number of plants emerged within each plot.
[c] Injury rated on a scale from 0 to 100%, with 100 representing plant death.
[d] Plant vigor rated on a scale from 1 to 5, with 5 being the most vigorous.
[e] Plant height measured from the ground to the top of the central stem.
[f] (*) Denotes measurements significantly different than the nontreated check. The nontreated check had a plant height of 23 cm and a fresh weight of 20 g $tray^{-1}$.

Table 2. The interaction effect of application timing and dicamba rate on soybean offspring emergence, observable injury, vigor, and relative yield. The main effect of application timing on the percentage of injured plants.[a] Offspring seed was collected from HBK 4950LL soybean cultivar. In 2014 parent plants were treated at Marianna, AR. Offspring plants were evaluated in a field trial in Marianna, AR in 2015. Data were collected from offspring at the second trifoliate stage.

Parent app. timing	Rate	Offspring emergence[b]	Injured plants[cd]	Injury[e]	Vigor[f]	Relative Yield[b]
	$g\ ae\ ha^{-1}$	——————%——————				%
V4	2.18	96 b	55 de	10 d	5 a	100 a
	8.75	100 a		16 d	5 b	100 a
V6	2.18	100 a	45 e	16 d	5 ab	100 a
	8.75	100 a		24 cd	5 ab	94 ab
R1	2.18	93 bc	49 e	15 d	5 a	100 a
	8.75	92 bc		21 d	5 a	98 ab
R2	2.18	88 bc	67 cd	19 d	5 a	99 ab
	8.75	92 bc		26 bcd	5 a	95 ab
R3	2.18	94 bc	57 de	18 d	5 a	100 a
	8.75	87 bc		18 d	5 ab	98 ab
R4	2.18	91 bc	75 bc	29 bc	4.5 bc	92 ab
	8.75	67 d*		75 a	3.5 d	95 ab
R5	2.18	84 c*	96 a	73 a	4 cd	100 a
	8.75	61 d*		83 a	2.25 f	92 ab
R6	2.18	93 bc	89 ab	43 b	4.25 c	97 ab
	8.75	86 bc		80 a	2.75 e	86 b

[a] Means in a column with the same letter do not differ according to Fisher's protected LSD at $\alpha=0.05$.

[b] Percent calculated from comparison of nontreated check. The nontreated check had an emergence of 181 plants and yielded 2820 kg ha^{-1}. (*) Denotes measurements significantly different than the nontreated check.

[c] Data averaged over two dicamba rates (2.18 g ae ha^{-1}, 8.75 g ae ha^{-1}).

[d] Percent calculated from the number of plants emerged within each plot.

[e] Injury rated on a scale from 0 to 100%, with 100 representing plant death.

[f] Plant vigor rated on a scale from 1 to 5, with 5 being the most vigorous.

Table 3. The main effect of application timing on soybean offspring emergence and fresh weight of aboveground biomass. The interaction effect of application timing and dicamba rate on the percentage of injured plants, observable injury, vigor, and plant height.[a] Offspring seed was collected from HALO 5.45LL soybean cultivar. In 2014 and 2015 parent plants were treated at Marianna, AR. Offspring plants were evaluated in a greenhouse at Fayetteville, AR in 2015 and 2016. Data were collected from offspring at the second trifoliate stage.

Parent app. timing	Rate	Offspring emergence[bc]		Injured plants[d]		Injury[e]		Vigor[f]		Plant height[cg]		Fresh weight[bc]	
	g ae ha^{-1}	—%		—%		—%				cm		g tray^{-1}	
V4	2.18	96	ab	1	f	1	f	5	a	28	a	21	a
	8.75			5	ef	2	f	5	a	27	a		
V6	2.18	96	ab	3	ef	1	f	5	a	28	a	19	abc
	8.75			6	ef	2	f	5	a	28	a		
R1	2.18	98	ab	12	ef	4	f	5	a	28	a	19	ab
	8.75			18	e	4	f	5	a	27	a		
R2	2.18	100	a	10	ef	6	f	5	a	27	a	21	a
	8.75			8	ef	5	f	5	a	26	a		
R3	2.18	92	bc	13	ef	7	f	4.5	ab	25	ab	18	bc
	8.75			43	d	21	e	3	cd	20	b		
R4	2.18	86	c*	44	cd	21	de	3	d	19	b*	15	d*
	8.75			68	ab	34	bc	3	de	17	bc*		
R5	2.18	92	bc	45	cd	21	de	4	bc	19	b*	16	cd*
	8.75			76	ab	43	ab	2	ef	18	bc*		
R6	2.18	97	ab	61	bc	29	cd	3	d	19	b*	17	bcd*
	8.75			84	a	47	a	2	f	14	c*		

[a] Means in a column with the same letter do not differ according to Fisher's protected LSD at α=0.05.

[b] Data averaged over two dicamba rates (2.18 g ae ha^{-1}, 8.75 g ae ha^{-1}).

[c] Percent calculated from comparison of nontreated check. The nontreated check had an emergence of 10 plants, a plant height of 27 cm, and a fresh weight of 22 g tray^{-1}. (*) Denotes measurements significantly different than the nontreated check.

[d] Percent calculated from the number of plants emerged within each plot.

[e] Injury rated on a scale from 0 to 100%, with 100 representing plant death.

[f] Plant vigor rated on a scale from 1 to 5, with 5 being the most vigorous.

[g] Plant height measured from the ground to the top of the central stem.

Table 4. The interaction effect of application timing and dicamba rate on soybean offspring emergence, observable injury, vigor, and the main effect of application timing on the percentage of injured plants.[a] Offspring seed was collected from HALO 5.45LL soybean cultivar. In 2014, parent plants were treated at Marianna, AR. Offspring plants were evaluated in a field trial at Marianna, AR in 2015. Data were collected from offspring at the second trifoliate stage.

Parent app. timing	Rate	Offspring emergence[b]		Injured plants[d]		Injury[e]		Vigor[f]		Relative yield[b]	
	g ae ha^{-1}	——%				———				%	
V4	2.18	100	a	14	f	6	f	5	a	90	ab
	8.75	100	a			12	ef	5	a	84	ab
V6	2.18	100	ab	24	ef	8	f	5	ab	90	ab
	8.75	82	d			13	ef	4.25	bc	94	ab
R1	2.18	96	abc	30	def	9	f	4.5	abc	86	ab
	8.75	81	d			19	def	4.5	abc	90	ab
R2	2.18	90	bcd	32	de	14	ef	4.75	ab	94	ab
	8.75	88	cd			20	cdef	4.75	ab	92	ab
R3	2.18	96	abc	43	cd	20	cdef	4.75	ab	96	ab
	8.75	68	ef*			36	c	3.5	de	83	ab
R4	2.18	98	abc	59	bc	28	cde	4	cd	99	a
	8.75	76	de*			60	b	3.25	e	82	ab
R5	2.18	89	cd	84	a	59	b	3.5	e	86	ab
	8.75	57	f*			88	a	1.75	f	82	ab
R6	2.18	100	a	62	b	34	cd	5	a	87	ab
	8.75	100	abc			65	b	3.25	e	80	b

[a] Means in a column with the same letter do not differ according to Fisher's protected LSD at α=0.05.

[b] Percent calculated from comparison of nontreated check. The nontreated check had an offspring emergence of 203 plants and yielded 3220 kg ha^{-1}. (*) Denotes measurements significantly different than the nontreated check.

[c] Data averaged over two dicamba rates (2.18 g ae ha^{-1}, 8.75 g ae ha^{-1}).

[d] Percent calculated from the number of plants emerged within each plot.

[e] Injury rated on a scale from 0 to 100%, with 100 representing plant death.

[f] Plant vigor rated on a scale from 1 to 5, with 5 being the most vigorous.

Table 5. The main effect of parent application timing on soybean offspring emergence and the interaction effect of application timing and dicamba rate on observable injury to soybean offspring, vigor, and percentage of malformed pods.[ab] Offspring seed was collected from HBK 4950 and HALO 5.45 soybean cultivars. In 2014 parent plants were treated at Rohwer, AR and Marianna, AR. Offspring was evaluated in a greenhouse at Fayetteville, AR in 2015. Visual injury and vigor ratings were collected at the second trifoliate stage. Soybean pods were collected at full maturity.

Parent app. timing	Rate	Offspring emergence[cd]		Injury[e]		Vigor[f]		Malformed pods	
	g ae ha^{-1}	——%		——				%	
V4	2.18	100	a	1	i	5	a	19	d
	8.75			1	i	5	a	21	d
V6	2.18	89	bc	3	hi	5	a	19	d
	8.75			2	hi	5	ab	19	d
R1	2.18	100	a	4	ghi	5	ab	24	bcd
	8.75			6	fghi	5	a	30	bc
R2	2.18	76	cd	2	hi	4	ab	23	cd
	8.75			9	fg	4	c	30	bc
R3	2.18	89	bc	8	fghi	4	ab	23	cd
	8.75			17	de	4	c	30	bc
R4	2.18	57	d*	13	ef	4	bc	25	bcd
	8.75			22	cd	3	d	43	a
R5	2.18	37	e*	39	b	3	de	23	cd
	8.75			57	a	2	f	24	cd
R6	2.18	95	ab	30	c	4	c	19	d
	8.75			53	a	2	ef	20	d

[a] Means in a column with the same letter do not differ according to Fisher's protected LSD at α=0.05.

[b] Data averaged over three pod locations (top, middle, bottom).

[c] Data averaged over two dicamba rates (2.18 g ae ha^{-1}, 8.75 g ae ha^{-1}).

[d] Percent calculated from comparison of nontreated check. The nontreated check had an offspring emergence of 10 plants. (*) Denotes measurements significantly different than the nontreated check.

[e] Injury rated on a scale from 0 to 100%, with 100 representing plant death.

[f] Plant vigor rated on a scale from 1 to 5, with 5 being the most vigorous.

Table 6. The main effect of pod position on soybean offspring emergence, visual injury, vigor, plant height, and fresh weight of aboveground biomass.[ab] Offspring seed was collected from HBK 4950LL and HALO 5.45LL soybean cultivars. In 2014 parent plants were treated at Rohwer, AR and Marianna, AR. Offspring plants were evaluated in a greenhouse at Fayetteville, AR. Data were collected from offspring at the second trifoliate stage. Pods were collected from parent plants at maturity.

Parent pod position	Offspring Emergence[c]		Injury[d]		Vigor[e]		Plant height[f]		Fresh weight		Malformed pods	
	——————%——————						cm		g tray^{-1}		%	
Top	83	a	16	b	4	a	19	a	22	a	25	b
Middle	79	ab	15	b	4	a	17	b	22	a	28	a
Bottom	70	b	22	a	3	b	14	c	9	b	20	b

[a] Means in a column with the same letter do not differ according to Fisher's protected LSD at α=0.05.

[b] Data averaged over eight application timings (V4, V6, R1-R6) and two dicamba rates (2.18 g ae ha^{-1}, 8.75 g ae ha^{-1}).

[c] Percent calculated from comparison of nontreated check. The nontreated check for the top pod position had an emergence of 11 plants, with a plant height of 20 cm, and a fresh weight of 32 g tray^{-1}, the middle pod position had an emergence of 10 plants, with a plant height of 18 cm, and a fresh weight of 29 g tray^{-1}, and the bottom pod position had an emergence of 8 plants, with a plant height of 15 cm, and a fresh weight of 14 g tray^{-1}.

[d] Injury rated on a scale from 0 to 100%, with 100 representing plant death.

[e] Plant vigor rated on a scale from 1 to 5, with 5 being the most vigorous.

[f] Plant height measured from the ground to the top of the central stem.

GENERAL CONCLUSIONS

Grain yield of soybean is highly sensitive to dicamba during the late vegetative/early reproductive growth stages, and observable injury is at best a moderate indicator of yield loss. Damage to the soybean terminal, resulting in reduced plant height, appears to be a good indicator of yield reduction; however, other factors influence height reductions such as application timing and cultivar selection. Unpredictable yield loss in soybean from dicamba is attributed to application timing, but the plasticity of soybean makes it difficult to generalize the effects of dicamba. Furthermore, greater recovery was observed from late-maturing varieties due to vegetative growth remaining for a longer period before flowering; however, a determinate soybean may be more sensitive to exposure to dicamba during vegetative growth stages compared to an indeterminate soybean.

The implication that dicamba can be transported to the seed of susceptible cultivars could have significant impacts on producers who grow seed of conventional or other transgenic lines that are not resistant to the dicamba herbicide. As the adoption of dicamba technology increases, it will be important for growers to follow a stringent application program to decrease the potential of off-target movement of this herbicide. In addition, it appears that utilizing dicamba formulations that are less volatile will be necessary to further reduce potential for off-target movement.