Effect of soybean growth stage on sensitivity to sublethal rates of dicamba and 2,4-D

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Abstract

Field experiments were conducted in 2012 and 2013 across four locations for a total of 6 site-years in the midsouthern United States to determine the effect of growth stage at exposure on soybean sensitivity to sublethal rates of dicamba (8.8 g ae ha^{-1}) and 2,4-D (140 g ae ha^{-1}). Regression analysis revealed that soybean was most susceptible to injury from 2,4-D when exposed between 413 and 1,391 accumulated growing degree days (GDD) from planting, approximately between V1 and R2 growth stages. In terms of terminal plant height, soybean was most susceptible to 2,4-D between 448 and 1,719 GDD, or from V1 to R4. However, maximum susceptibility to 2,4-D was only between 624 and 1,001 GDD or from V3 to V5 for yield loss. As expected, soybean was sensitive to dicamba for longer spans of time, ranging from 0 to 1,162 GDD for visible injury or from emergence to R2. Likewise, soybean height was most affected when dicamba exposure occurred between 847 and 1,276 GDD or from V4 to R2. Regarding grain yield, soybean was most susceptible to dicamba between 820 and 1,339 GDD or from V4 to R2. Consequently, these data indicate that soybean response to 2,4-D and dicamba can be variable within vegetative or reproductive growth stages; therefore, specific growth stage at the time of exposure should be considered when evaluating injury from off-target movement. In addition, application of dicamba near susceptible soybean within the V4 to R2 growth stages should be avoided because this is the time of maximum susceptibility. Research regarding soybean sensitivity to 2,4-D and dicamba should focus on multiple exposure times and also avoid generalizing growth stages to vegetative or reproductive.

Transgenic crops resistant to 2,4-D or dicamba have been widely adopted in the United States and are projected to increase in hectarage (Mortensen et al. 2012). Despite decades of 2,4-D and dicamba use in monocot crops and noncrop areas, transgenic cultivars have allowed for wider use patterns (Mortensen et al. 2012). Consequently, increased off-target movement and resultant injury to susceptible soybean have been reported since the introduction of resistant cultivars (EPA 2017). Spray drift, vapor drift, sprayer contamination, and misapplication have been identified as common modes of off-target 2,4-D and dicamba movement to susceptible soybean (Behrens and Lueschen 1979; Cundiff et al. 2017; Egan and Mortensen 2012; Grover et al. 1972; Johnson et al. 2012; Soltani et al. 2016; Steckel et al. 2010; Strachan et al. 2013). In addition, it has been speculated that injury symptoms from 2,4-D and dicamba, which are unique and conspicuous, even after low-dose exposure, are reported more often than off-target movement of other herbicides, because they are more easily recognized (Sciumbato et al. 2004).

Differential sensitivity of soybean to 2,4-D and dicamba has been well characterized. On an equivalent-rate basis, soybean is far more sensitive to dicamba than to 2,4-D; however, sensitivity often depends on growth stage at the time of exposure (Andersen et al. 2004; Egan et al. 2014; Johnson et al. 2012; Kelley et al. 2005; Robinson et al. 2013a, 2013b; Sciumbato et al. 2004; Wax et al. 1969). Sublethal rates of 2,4-D and dicamba can cause alarming levels of soybean injury; however, soybean exposure to dicamba often results in a greater yield loss than exposure to 2,4-D (Egan et al. 2014; Johnson et al. 2012; Kelley et al. 2005; Wax et al. 1969). Dicamba more frequently causes soybean apical meristem death than does 2,4-D (Kelley et al. 2005). Furthermore, growth stage at the time of soybean exposure to 2,4-D or dicamba and subsequent apical meristem death determines the extent of branching and subsequent reproductive growth at lower nodes (Carpenter and Board 1997; Kelley et al. 2005; Robinson et al. 2013b; Wax et al. 1969). Consequently, the magnitude of soybean yield loss from 2,4-D or dicamba can be influenced by the time of exposure in relation to apical meristem death.

Table 1. Details of experiments in which time of sublethal 2,4-D or dicamba exposure were evaluated on susceptible soybean.

Year	Location	Coordinates	Soil series	Planting date	Variety[a,b]	Seeding rate[c]
2012	Starkville, MS	33°47′60.9″N, 88°77′38.1″W	Leeper silty clay[d]	May 15	AG 4932	345,940
2012	Brooksville, MS	33°15′24.5″N, 88°33′24.4″W	Brooksville silty clay[e]	May 1	AG 4932	345,940
2013	Starkville, MS	33°47′60.9″N, 88°77′38.1″W	Leeper silty clay[d]	May 30	PKP 95Y61*	340,998
2013	Brooksville, MS	33°15′24.5″N, 88°33′24.4″W	Brooksville silty clay[e]	May 22	PKP 95731*	345,940
2013	Stoneville, MS	33°44′00.9″N, 90°88′59.9″W	Tunica clay[f]	May 16	PKP 94Y82	345,940
2013	Rohwer, AR	33°80′72.8″N, 91°27′43.7″W	Herbert silt loam[g]	June 25	HBK 4950	321,230

[a] AG 4932, Asgrow, Monsanto Company, St. Louis, MO; PKP 95Y61, PKP 95731, PKP 94Y82, Pioneer Hi-Bred International, Inc., Johnston, IA; HBK 4950 Bayer CropScience, Research Triangle Park, NC.
[b] Asterisk indicates determinate variety.
[c] Seeding rates are expressed in seeds ha^{-1}.
[d] Soil organic matter, 2.4%; pH, 5.9; taxonomic class, fine, smectitic, nonacid, thermic vertic epiaquepts.
[e] Soil organic matter, 1.6%; pH, 7.2; taxonomic class, fine, smectitic, thermic Aquic Hapluderts.
[f] Soil organic matter, 2.2%; pH, 6.7; taxonomic class, clayey over loamy, smectitic over mixed, superactive, nonacid, thermic vertic epiaquepts.
[g] Soil organic matter, 2.2%; pH, 7.1; taxonomic class, fine-silty, mixed, superactive, mesic udollic epiaqualfs.

Before a universal soybean growth-stage description system was developed, many researchers evaluated herbicide sensitivity at growth stages characterized in relation to bloom. For example, Wax et al. (1969) and Weidenhamer et al. (1989) evaluated soybean response to dicamba and 2,4-D exposure at prebloom and midbloom. However, even after Fehr and Caviness (1977) described soybean growth stages, researchers still commonly only tested soybean response to 2,4-D or dicamba exposure at one or two vegetative or reproductive growth stages. Customarily, soybean sensitivity to 2,4-D and dicamba has been tested at an early vegetative stage (V2 to V4) and an early reproductive stage (R1 or R2) (Al-Khatib and Peterson 1999; Anderson et al. 2004; Auch and Arnold 1978; Egan et al. 2014; Griffin et al. 2013; Robinson et al. 2013a, 2013b; Solomon and Bradley 2014; Soltani et al. 2016). Egan et al. (2014) reported a meta-analysis on 2,4-D and dicamba effects on soybean and cotton (*Gossypium hirsutum* L.). Of the 30 studies reviewed from the last seven decades, they concluded that dicamba was more likely to cause soybean yield loss when exposure occurred at reproductive growth stages rather than at vegetative growth stages; however, soybean response to 2,4-D was similar across all growth stages (Egan et al. 2014). Egan et al. (2014) also noted that soybean exposure to 2,4-D at pod-development stages was not reported in any of the studies. Furthermore, reports of soybean response to 2,4-D are conflicting; Robinson et al. (2013a) determined yield loss was greater at R2 than at vegetative growth stages, whereas Kelley et al. (2005) reported greater yield losses at V3 than R2.

Evidence in the literature supports our hypothesis that differential sensitivity to herbicides exists within vegetative or reproductive growth stages. For example, soybean is more sensitive to acifluorfen and naptalam plus dinoseb at V3 than at V5 (Kapusta et al. 1986). Also, Robinson et al. (2013a) reported soybean was more sensitive to 2,4-D at V5 than at V2, and Kelley et al. (2005) reported lower soybean yields after 2,4-D exposure at V3 than at V7. Likewise, Robinson et al. (2013b) and Auch and Arnold (1978) reported soybean yield was more sensitive to dicamba exposure at early vegetative rather than later vegetative growth stages. However, Kelley et al. (2005) reported soybean yield sensitivity to dicamba at V3 versus V7 was rate dependent. Ultimately, to our knowledge, no published experiment has investigated soybean response to 2,4-D or dicamba across all growth stages. Off-target movement of 2,4-D or dicamba can occur at any time; therefore, it is important to understand soybean response at all stages.

Current literature indicates that soybean growth stage plays a major role in soybean sensitivity to 2,4-D and dicamba. However, little is known about soybean response to these herbicides at every growth stage under similar environmental conditions. Accordingly, the objectives of this research were to determine sensitivity of soybean to sublethal rates of 2,4-D and dicamba across all growth stages and to predict the times of soybean maximum susceptibility to 2,4-D and dicamba.

Materials and Methods

Field experiments were conducted in 2012 and 2013 to determine the effect of soybean growth stage at exposure on sensitivity to 2,4-D or dicamba. All experiments were conducted under conventional tillage on raised beds. Plots were 3.9-m wide by 12.2-m long, using four, 97-cm spaced rows. Years, locations, coordinates, soil information, planting dates, varieties, and seeding rates are given in Table 1.

Each herbicide (2,4-D and dicamba) was tested in separate experiments, using a randomized complete block design with four replications. All herbicide treatments were applied using a CO_2-pressurized backpack sprayer equipped with a shielded hand-held spray boom calibrated to deliver a spray volume of 140 L ha^{-1} at 241 kPa (XR 8002 nozzles in 2012) or 207 kPa (TTI 11002 nozzles in 2013) (TeeJet Technologies, Spraying Systems Co., Wheaton, IL). Treatments were applied to the center two rows of each plot, leaving the outside two rows to act as a buffer in preventing contamination of adjacent plots. All fields were maintained weed free for the duration of experiments with broadcast applications of glyphosate and hand weeding to avoid weed competition and interference. Fertility and pest control practices at all sites were conducted as needed according to recommended local standard production practices.

A single sublethal rate of dicamba (8.8 g ae ha^{-1}) (Clarity, BASF Corp., Research Triangle Park, NC) or 2,4-D (140 g ae ha^{-1}) (Weedar 64, Nufarm Inc., Alsip IL) was applied weekly starting 1 wk after emergence through physiological maturity and growth stage of the majority (>50%) of the plot at application was recorded (Fehr and Caviness 1977). According to previous research, herbicide rates chosen in the current experiments were similar to rates possible through particle drift of dicamba and application error or tank contamination for 2,4-D (Brown et al. 2004; Carlsen et al. 2006; Egan et al. 2014). However, because sublethal rates of 2,4-D or dicamba were applied at a constant carrier volume in these experiments, spray coverage, droplet retention, and herbicide solution concentration would be more comparable to a tank contamination exposure rather than to spray drift or vapor exposure (Banks and Schroeder 2002; Ellis et al. 2002; Smith et al. 2017). A higher rate of 2,4-D than dicamba was chosen to ensure soybean response would be high enough to observe the effect of growth stage (Egan et al. 2014; Sciumbato et al. 2004; Solomon and

Bradley 2014). In addition to herbicide treatments, all experiments included a nontreated control (NTC) for comparison purposes.

Data Collection and Analysis

Visible evaluations of soybean injury were conducted 7, 14, 21, and 28 d after treatment (DAT) on the basis of a 0 (no symptoms) to 100 (plant death) scale similar to the one proposed by Sciumbato et al. (2004). In addition, heights of six random plants per plot were measured immediately before harvest. At physiological maturity, the center two rows of each plot were machine harvested using a small plot combine and yields were adjusted to 13% moisture. Soybean terminal height and grain yield data were then converted to percentage of the NTC by dividing data by the NTC of the respective block and multiplying by 100.

Because of the differential duration of soybean growth stages, some treatments occurred multiple times per block, because treatments were applied weekly, which resulted in an unbalanced data set. Therefore, data were first subjected to mixed-model ANOVA, and the LSMEANS statement (SAS, version 9.4; SAS Institute, Cary, NC) at α = 0.05 was used to detect treatment means different from the NTC, using the PROC GLIMMIX procedure in SAS. Because of the different nozzles used between years, the main effect and associated interactions of site-year were first considered fixed effects in the ANVOA model to test for significance. However, no interactions between site-year and growth stage were detected (P ≥ 0.13). Consequently, site-year and block (nested within site-year) were considered random effects, and treatment (growth stage at application) was considered a fixed effect in the ANOVA model to allow for inferences across multiple environmental conditions (Blouin et al 2011; Carmer et al. 1989; Yang 2010). Furthermore, all data were arcsine–square root transformed and subjected to ANOVA. However, results from transformed data were not different from original (nontransformed) data; therefore, original data were used in analyses.

To further characterize the effect of exposure time, accumulated growing degree days (GDD) from the planting date were calculated for each treatment at each site-year to provide a continuous numeric explanatory variable for regression analysis. Air temperature data were collected by either on-site weather stations or by nearby university-sponsored weather data collectors (Mississippi State University Extension 2018). GDDs were then calculated using the following formula:

$$GDD = \left[\sum (T_{max} + T_{min})/2 \right] - T_b$$

where T_{max} is the maximum daily air temperature, T_{min} is the minimum daily air temperature, and T_b is the base threshold temperature, which was set to 10 C, similar to Sbatella et al. (2016). Data were then fitted over GDD using several parametric regression models; however, the models evaluated did not adequately describe trends in the data. Therefore, data were regressed using nonparametric local regression fitted with a 95% confidence band (CB), using the loess package in R (version 3.5.1, RStudio Inc, Boston, MA). Points of interest in the local regression and their respective 95% confidence intervals (CIs) were predicted using the stats and base packages in R.

Results and Discussion

Soybean Response to 2,4-D

At 7 DAT, soybean treated with 2,4-D at R5, R6, R6.5, or R7 did not have injury levels higher than the NTC (Supplementary Table 1).

However, regression analysis revealed that soybean injury 7 DAT increased with delayed exposure timing from planting until 413 GDD, at which time soybean was most sensitive to 2,4-D until exposure timing reached 1,262 GDD (Figure 1). This period of maximum susceptibility approximately correlated to between V1 and R2 growth stages. After 1,262 GDD, soybean injury decreased as exposure time was delayed until 2,965 GDD (approximately R7 growth stage), when the 95% CB reached 0% injury. By 14 DAT, 2,4-D treatment at V1, V5, or R5.5 resulted in soybean injury levels similar to the NTC (Supplementary Table 1). Soybean injury 14 DAT was highest when exposure occurred from 624 to 1,291 GDD, which was approximately between the V3 and R2 growth stages. Kelley et al. (2005) reported approximately 32% higher soybean injury 14 DAT from 2,4-D at 180 g ae ha⁻¹ than we measured in the present experiment when plants were treated at V3; however, injury from treatment at R2 reported by Kelley et al. (2005) was similar to that measured in the present experiment. Regression of soybean injury 14 DAT displayed a similar trend to the 7 DAT data for 2,4-D exposure (Figure 1).

Soybean treated with 2,4-D at VC, V2, V4, V5, V6, R1, R2, R3, or R4 resulted in 13% to 18% injury 21 DAT (Supplementary Table 1). Likewise, regression analysis indicated a period of maximum susceptibility to 2,4-D at between 615 and 1,391 GDD, which was approximately between V3 and R2 growth stages (Figure 1). In addition, in terms of injury 21 DAT, soybean was least susceptible to 2,4-D exposure after 2,478 GDD or approximately the R5 growth stage. By 28 DAT, soybean injury from 2,4-D did not differ from the injury to NTC when treated at V1, R5, or R5.5 (Supplementary Table 1). However, treatment at R6, R6.5, or R7 was not evaluated, because plants had either senesced or were harvested by 28 DAT. Interestingly, Robinson et al. (2013a) used nonlinear regression to predict the effective dose needed to cause 20% soybean injury 28 DAT from 2,4-D. Their model calculated an effective dose of 145 g ae ha⁻¹ for soybean treated at V5. In the present experiment, treatment with 2,4-D at 140 g ae ha⁻¹ at V5 resulted in 20% injury, indicating that these data were quite similar (Supplementary Table 1). In the present experiment, regression of soybean injury 28 DAT indicated a period of maximum susceptibility between 669 and 1,290 GDD (approximately V3 to R2) and that soybean injury was minimal when treated at 2,322 GDD (approximately R5) or later (Figure 1). Despite differences in maximum values, all regressions from soybean injury from 2,4-D followed the same trend of increasing injury from planting until the maximum (17% to 22% injury) and then decreasing in injury until late reproductive growth stages (R5 to R7), when injury was similar to 0% (Figure 1). Considering all evaluation intervals, soybean was most sensitive to 2,4-D between V1 and R2 growth stages in terms of injury.

The magnitude of soybean height or yield reductions from 2,4-D was much lower than earlier evaluated injury (Figure 1). However, the period of maximum susceptibility to 2,4-D was much longer for soybean height response compared with injury, ranging from 448 to 1,719 GDD or from V1 to R4 growth stages, despite a minimum value of 92% of the NTC (Figure 1). Soybean yield affected by 2,4-D was relatively static across exposure times (Figure 1). Because of the low response of soybean to 2,4-D, the 95% CB of the regression line overlapped both minimum yield and 100% of the NTC lines for most exposure timings. As noted in Figure 1, soybean yield response was similar to the minimum value when exposure occurred from planting to R2 and from R6 to harvest. However, if exposure occurred between 624 and 1,001 GDD (approximately V3 to V5), soybean yield was lower
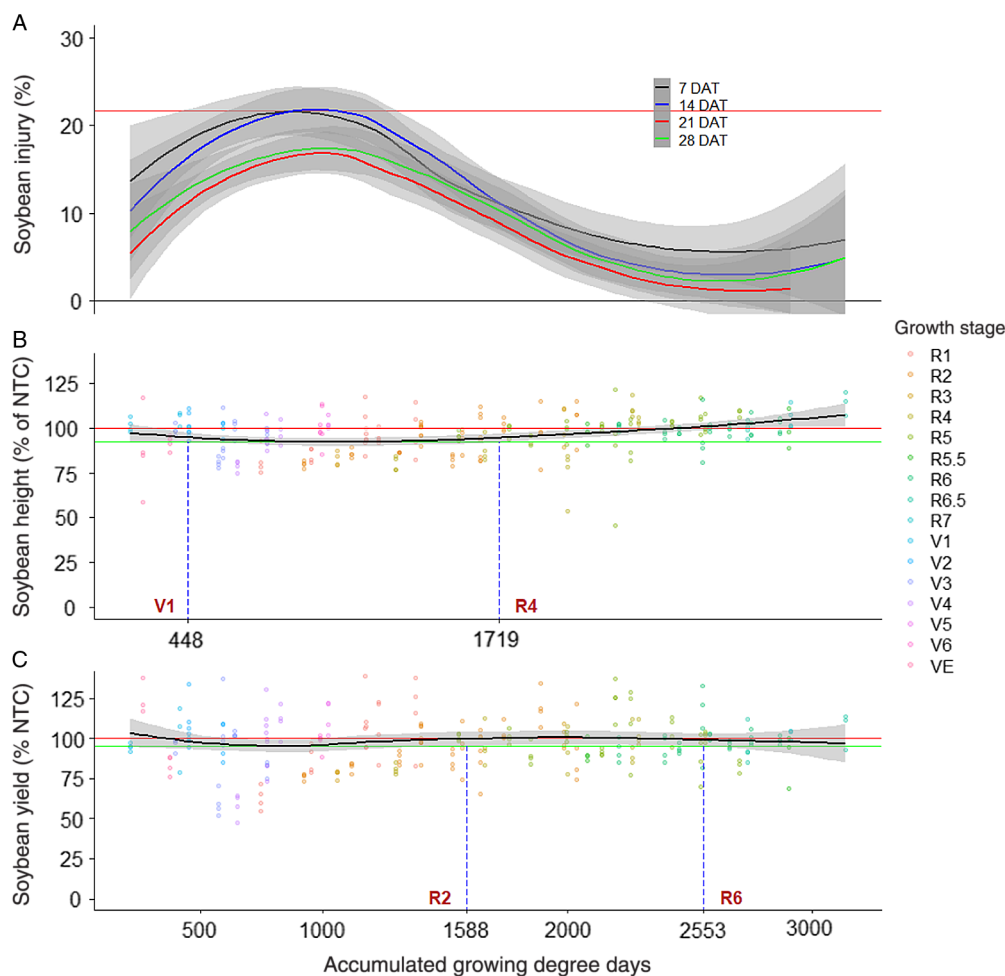
Figure 1. Nonparametric local regression of (A) soybean injury 7, 14, 21, and 28 d after treatment (DAT), (B) soybean terminal height, and (C) soybean grain yield after treatment with 2,4-D at 140 g ae ha^{-1}, as affected by time of exposure expressed in accumulated growing degree days (base temperature, 10 C) from planting across 6 site-years in Mississippi and Arkansas in 2012 and 2013. Grey bands represent 95% confidence. Horizontal red lines signify maximum injury, height, or yield of the fitted line. Horizontal green lines signify minimum height or yield of the fitted line. Vertical, dashed blue lines signify points where the 95% confidence band of the fitted line departs from minimum of the fitted line. NTC, nontreated control.

than 100% of the NTC. Research conducted in the 1950s and 1960s indicated that in terms of yield, soybean was more sensitive to 2,4-D at reproductive growth stages than at vegetative growth stages (Slife 1956; Wax et al. 1969). However, our results do not agree. This difference in results may have been due to the change in soybean cultivars over the past 50 years, sublethal rate used, or environmental conditions during experiments (Griffin et al. 2013; Kahlon et al. 2011).

Robinson et al. (2013a) reported that soybean injury from 2,4-D was highly correlated with yield, and they used regression analysis to predict yield based on injury. However, our data do not support using injury or plant height responses to 2,4-D as a direct predictor for yield. For example, soybean injury from 2,4-D exposure at most vegetative growth stages was consistently higher than the NTC across all evaluations (Supplementary Table 1). However, all vegetative growth-stage exposure timings except V3 resulted in soybean yield similar to the NTC. A possible cause for the lack of yield response observed in the present experiment was that only a single sublethal rate was used, whereas Robinson et al. (2013a) developed regression models based on 10 rates at three growth stages. This speaks to the effect of herbicide rate on plant response. For example, Kelley et al. (2005) found no soybean yield loss from 2,4-D treatment at V3 at 56 g ae ha^{-1};

however, a yield loss was observed when the rate was increased to 180 g ae ha^{-1}. Conversely, 2,4-D applied at 56 g ae ha^{-1} has been shown to reduce soybean plant height and yield when applied at V3, V7, or R2 and V7 or R2, respectively (Kelley et al. 2005). However, data from Kelley et al. (2005) were generated in Illinois, which likely experienced much different environmental conditions compared with our experiment, which was conducted in the midsouthern United States. Consequently, environmental conditions such as lower soil-moisture content could have promoted higher sensitivity to 2,4-D for Kelley et al. (2005) (Anderson et al. 2004; Auch and Arnold 1978; Griffin et al. 2013; Kelley et al. 2005; Weidenhamer et al. 1989). It is also important to note that 2,4-D exposure at V3 was the only treatment for which yield loss was observed, and many experiments have used V3 as the representative stage for all vegetative growth stages. This could be misleading, because 2,4-D exposure at V3 is not equivalent to other vegetative stages.

The number of observations was highest for the R2 growth stage ($n = 68$), suggesting that this growth stage was the longest across experimental sites. This is particularly concerning, because soybean exposure to 2,4-D at R2 was within the period of maximum susceptibility for all injury evaluation timings (Figure 1). However, soybean yield was similar to that of the
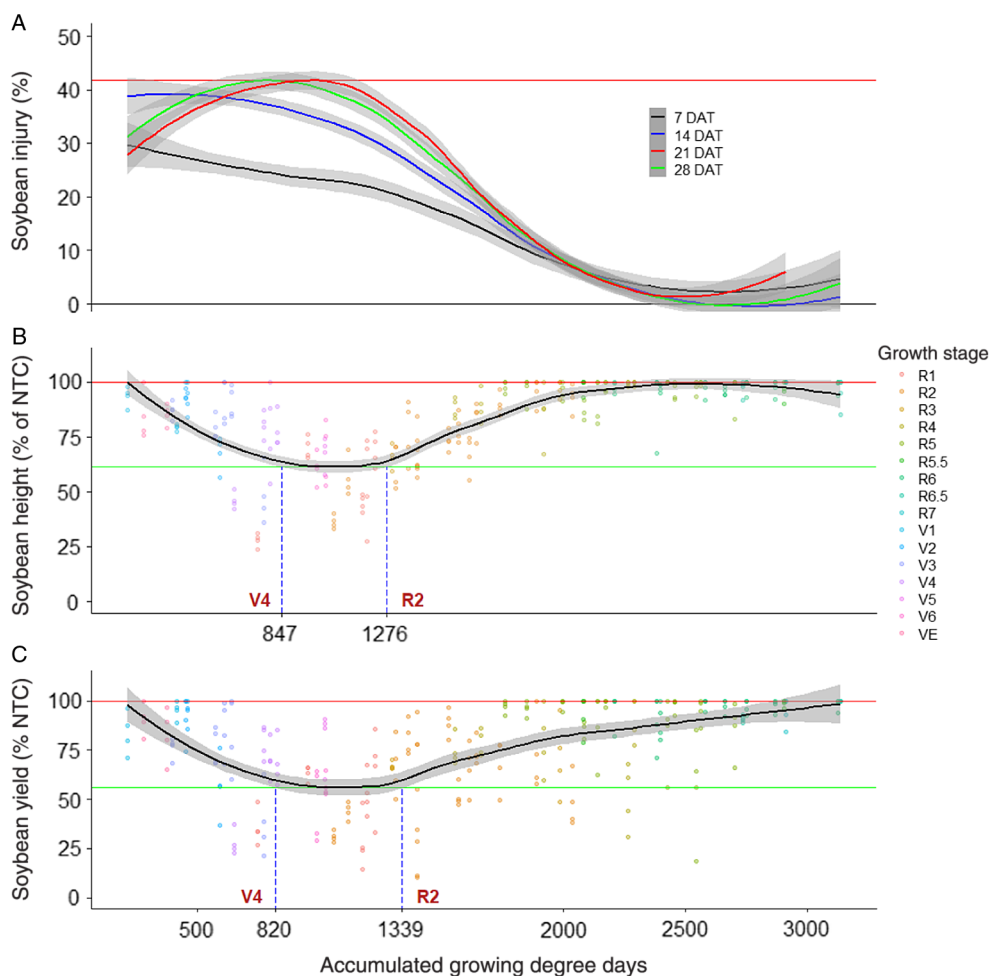
Figure 2. Nonparametric local regression of (A) soybean injury 7, 14, 21, and 28 d after treatment (DAT), (B) soybean terminal height, and (C) soybean grain yield after treatment with dicamba at 8.8 g ae ha^{-1}, as affected by time of exposure expressed in accumulated growing degree days (base temperature, 10 C) from planting across 6 site-years in Mississippi and Arkansas in 2012 and 2013. Grey bands represent 95% confidence. Horizontal red lines signify maximum injury, height, or yield of the fitted line. Horizontal green lines signify minimum height or yield of the fitted line. Vertical, dashed blue lines signify points where the 95% confidence band of the fitted line departs from minimum of the fitted line. NTC, nontreated control.

NTC when 2,4-D exposure occurred at R2 (Figure 1; Supplementary Table 1). Consequently, soybean growers may be more likely to observe alarming injury levels from 2,4-D exposure at R2 despite minimal risk for yield loss at the rate tested.

Soybean Response to Dicamba

Regression of soybean injury 7 DAT resulted in a different trend than in the 2,4-D experiments, where injury started at the maximum and continually decreased (Figure 2). Consequently, the period of maximum susceptibility for soybean injury 7 DAT after dicamba treatment was only between 0 and 452 GDD, or approximately between emergence and V2. Likewise, soybean was least susceptible to dicamba injury 7 DAT when exposure occurred after 2,694 GDD or the R6 growth stage. Similarly, soybean injury 14 DAT also decreased immediately from the maximum value (Figure 2). Consequently, the period of maximum susceptibility was between 0 and 733 GDD, or from planting to V4. However, soybean was not susceptible to dicamba exposure after 2,394 GDD or the R5 growth stage.

By 21 DAT, the period of maximum susceptibility to dicamba shifted to between 556 and 1,031 GDD, or from V2 to R1, including

exposure times that were not within maximum susceptibility range during the 7 and 14 DAT evaluations (Figure 2). Likewise, soybean was least susceptible to dicamba exposure after 2,346 GDD or around the R5 growth stage. Minimal soybean injury 28 DAT was observed from dicamba treatment at R5 or R6; however, the R6.5 and R7 treatments were not evaluated 28 DAT, because of senescence or harvest. On the basis of soybean injury 28 DAT, soybean was most susceptible to dicamba between 703 and 1,162 GDD, or from V3 to R2, and least susceptible between 2,360 and 2,692 GDD, or from R5 to R6.5 (Figure 2). Unlike other evaluation intervals, soybean injury 28 DAT increased from 0% after 2,692 GDD. However, this increase was likely due to missing data at these stages, due to plots being harvested before the evaluation interval.

Soybean plant height was not affected by dicamba when treated at R4, R5, R5.5, R6, R6.5, or R7 (Supplementary Table 2). However, soybean height was most affected when dicamba exposure occurred between 847 and 1,276 GDD, or between V4 and R2 (Figure 2). Exposure during this period was similar to the minimum value of the fitted regression line, which was 61% of the NTC. However, dicamba exposure after 2,244 GDD, or R4, resulted in plant heights similar to those of the NTC. Griffin et al.

(2013) reported soybean plant height was more affected by dicamba applied at R1 than at V3/V4, when the dicamba rate was at least 17.5 g ae ha^{-1}.

No yield loss was observed from dicamba treatment at V1, R5, R5.5, R6, R6.5, or R7 (Supplementary Table 2). However, soybean yield was most affected when dicamba exposure occurred between 820 and 1,339 GDD, or from V4 to R2 (Figure 2). Likewise, soybean yield was similar to that of the NTC when exposed to dicamba from emergence to 272 GDD (<VC) and from 2,858 GDD (R6.5) to harvest. Unlike the 2,4-D experiment, yield losses observed from dicamba where highly reflective of injury and plant height observations, as can be confirmed from the regression analysis. For example, the period of maximum susceptibility of soybean to dicamba in terms of plant height was from 847 GDD (V4) to 1,276 GDD (R2) (Figure 2). Likewise, in terms of yield, the period of maximum susceptibility of soybean to dicamba was from 820 GDD (V4) to 1,339 GDD (R2) (Figure 2). Similarly, Griffin et al. (2013) found that soybean injury from dicamba treatment at V3/V4 resulted in proportionally similar yield reductions. Soybean yield loss from dicamba has been reported to be much greater when treated at "early/mid-bloom" than V2 to V4 (Auch and Arnold 1978; Wax et al. 1969). Likewise, from their meta-analysis, Egan et al. (2014) concluded that soybean yield was more affected by dicamba exposure at flowering stages than at vegetative or pod development. This commonly observed differential yield response of soybean to dicamba at early reproductive growth stages compared with early vegetative growth stages is likely due to damaged reproductive structures and apical meristem death that cannot be compensated for by branching, because injury occurs after vegetative growth rate has slowed (Carpenter and Board 1997; Griffin et al. 2013; Wax et al 1969). Conversely, Weidenhamer et al. (1989) found soybean yield loss was greater when exposed to dicamba at a prebloom vegetative growth stage than at a midbloom reproductive growth stage. Whereas Kelley et al. (2005) found no difference in yield after treatment with dicamba at 5.6 g ae ha^{-1}.

Similar to the 2,4-D experiment, the duration of R2 growth stage was longest in the dicamba experiment (n = 68 observations). Because of the duration of this growth stage and the significant yield loss observed from dicamba exposure at R2, caution should be exercised by dicamba applicators during this time. It is also important to note that previous literature has indicated soybean response to dicamba and 2,4-D can be highly inconsistent. Potential causes for variability in soybean response to herbicides identified in the literature include environmental conditions, herbicide rate, carrier volume, and soybean growth strategy (indeterminate or determinate) (Al-Khatib and Peterson 1999; Anderson et al. 2004; Auch and Arnold 1978; Banks and Schroeder 2002; Ellis et al. 2002; Kelley et al. 2005; Smith et al. 2017; Wax et al. 1969; Weidenhamer et al. 1989).

In summary, these data indicate that soybean response to 2,4-D and dicamba cannot be generalized to vegetative or reproductive growth stages, because specific growth stages must be accounted for. In considering the levels of yield loss observed and the rates tested, 2,4-D exposure to susceptible soybean likely poses less risk than dicamba; however, alarming levels of injury still can be observed from 2,4-D exposure, and higher rates may cause a wider range of susceptibility to yield loss. Dicamba applications near susceptible soybean between the stages of V4 and R2 should be avoided because this time span can be considered the time of maximum susceptibility for yield loss. Furthermore, great care in thoroughly cleaning spray equipment contaminated with dicamba should be taken if the same equipment must be used for any type of application to soybean within this stage range.

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References

Al-Khatib K, Peterson D (1999) Soybean (*Glycine max*) response to simulated drift from selected sulfonylurea herbicides, dicamba, glyphosate, and glufosinate. Weed Technol 13:264–270

Anderson SM, Clay SA, Wrage LJ, Matthees D (2004) Soybean foliage residues of dicamba and 2, 4-D and correlation to application rates and yield. Agron J 96:750–760

Auch DE, Arnold WE (1978) Dicamba use and injury on soybeans (*Glycine max*) in South Dakota. Weed Sci 26:471–475

Banks PA, Schroeder J (2002) Carrier volume affects herbicide activity in simulated spray drift studies. Weed Technol 16:833–837

Behrens R, Lueschen WE (1979) Dicamba volatility. Weed Sci 27:486–493

Blouin DC, Webster EP, Bond JA (2011) On the analysis of combined experiments. Weed Technol 25:165–169

Brown RB, Carter MH, Stephenson GR (2004) Buffer zone and windbreak effects on spray drift deposition in a simulated wetland. Pest Manag Sci 60:1085–1090

Carlsen SCK, Spliid NH, Svensmark B (2006) Drift of 10 herbicides after tractor spray application. 2. Primary drift (droplet drift). Chemosphere 64:778–786

Carmer SG, Nyquist WE, Walker WM (1989) Least significant differences for combined analysis of experiments with two or three-factor treatment designs. Agron J 81:665–672

Carpenter AC, Board JE (1997) Branch yield components controlling soybean yield stability across plant populations. Crop Sci 37:885–891

Cundiff GT, Reynolds DB, Mueller TC (2017) Evaluation of dicamba persistence among various agricultural hose types and cleanout procedures using soybean (*Glycine max*) as a bio-indicator. Weed Sci 65:305–316

Egan JF, Barlow KM, Mortensen DA (2014) A meta-analysis on the effects of 2, 4-D and dicamba drift on soybean and cotton. Weed Sci 62:193–206

Egan JF, Mortensen DA (2012) Quantifying vapor drift of dicamba herbicides applied to soybean. Environ Toxicol Chem 31:1023–1031

Ellis JM, Griffin JL, Jones CA (2002) Effect of carrier volume on corn (*Zea mays*) and soybean (*Glycine max*) response to simulated drift of glyphosate and glufosinate. Weed Technol 16:587–597

[EPA] Environmental Protection Agency (2017) Compliance Advisory: Crop Damage Complaints Related to Dicamba Herbicides Raising Concerns. https://www.epa.gov/sites/production/files/2017-07/documents/fifra-dicambacomplianceadvisory-201708.pdf. Accessed: April 2, 2018

Fehr WR, Caviness CE (1977) Stages of soybean development. Ames, IA: Cooperative Extension Service, Iowa State University

Griffin JL, Bauerle MJ, Stephenson DO, Miller DK, Boudreaux JM (2013) Soybean response to dicamba applied at vegetative and reproductive growth stages. Weed Technol 27:696–703

Grover R, Yoshida K, Maybank J (1972) Droplet and vapor drift from butyl ester and dimethylamine salt of 2, 4-D. Weed Sci 20:320–324

Johnson VA, Fisher LR, Jordan DL, Edminsten KE, Stewart AM, York AC (2012) Cotton, peanut, and soybean response to sublethal rates of dicamba, glufosinate, and 2, 4-D. Weed Technol 26:195–206

Kahlon CS, Board JE, Kang MS (2011) An analysis of yield component changes for new vs. old soybean cultivars. Agron J 103:13–22

Kapusta G, Jackson LA, Mason DS (1986) Yield response of weed-free soybeans (*Glycine max*) to injury from postemergence broadleaf herbicides. Weed Sci 34:304–307

Kelley KB, Wax LM, Hager AG, Riechers DE (2005) Soybean response to plant growth regulator herbicides is affected by other postemergence herbicides. Weed Sci 53:101–112

Mississippi State University Extension (2018) Delta Agricultural Weather Center. Customizable data report station. http://deltaweather.extension.msstate.edu/weather-station-result/. Accessed: November 3, 2018

Mortensen DA, Egan JF, Maxwell BD, Ryan MR, Smith RG (2012) Navigating a critical juncture for sustainable weed management. Bioscience 62:75–84

Robinson AP, Davis VM, Simpson DM, Johnson WG (2013a) Response of soybean yield components to 2, 4-D. Weed Sci 61:68–76

Robinson AP, Simpson DM, Johnson WG (2013b) Response of glyphosate-tolerant soybean yield components to dicamba exposure. Weed Sci 61:526–536

Sbatella GM, Kniss AR, Omondi EC, Wilson RG (2016) Volunteer corn (*Zea mays*) interference in dry edible bean (*Phaseolus vulgaris*). Weed Technol 30:937–942

Sciumbato AS, Chandler JM, Senseman SA, Bovey RW, Smith KL (2004) Determining exposure to auxin-like herbicides. I. Quantifying injury to cotton and soybeans. Weed Technol 18:1125–1134

Slife FW (1956) The effect of 2, 4-D and several other herbicides on weeds and soybeans when applied as post-emergent sprays. Weeds 4:61–68

Smith HC, Ferrell JA, Webster TM, Fernandez JV (2017) Cotton response to simulated auxin herbicide drift using standard and ultra-low carrier volumes. Weed Technol 31:1–9

Solomon CB, Bradley KW (2014) Influence of application timing and sublethal rates of synthetic auxin herbicides on soybean. Weed Technol 28:454–464

Soltani N, Nurse RE, Sikkema PH (2016) Response of glyphosate-resistant soybean to dicamba spray tank contamination during vegetative and reproductive growth stages. Can J Plant Sci 96:160–164

Steckel L, Chism C, Thompson A (2010) Cleaning plant growth regulator (PGR) herbicide out of field sprayers. https://extension.tennessee.edu/publications/Documents/W071.pdf. Accessed: April 1, 2018

Strachan SD, Ferry NM, Cooper TL (2013) Vapor movement of aminocyclopyrachlor, aminopyralid, and dicamba in the field. Weed Technol 27:143–155

Wax LM, Knuth LA, Slife FW (1969) Response of soybeans to 2, 4-D, dicamba, and picloram. Weed Sci 7:388–393

Weidenhamer JD, Triplett GB, Sobotka FE (1989) Dicamba injury to soybean. Agron J 81:637–643

Yang RC (2010) Towards understanding and use of mixed-model analysis of agricultural experiments. Can J Plant Sci 90:605–627