Glyphosate-Resistant Soybean Response to Micro-Rates of Three Dicamba-Based Herbicides

O. Adewale Osipitan, Jon E. Scott, and Stevan Z. Knezevic*

Core Ideas
• The impact of simulated dicamba drift on growth and yield of glyphosate-resistant soybean was similar among dicamba formulations.
• The impact of dicamba drift on soybean could be influenced by moisture condition of the environmental field.
• Late vegetative or early flowering stage of soybean was the most sensitive growth stage to dicamba drift.

ABSTRACT

New dicamba-based herbicides such as Engenia (N,N-bis-(3-aminopropyl) methylamine salt) and XtendiMax (diglycolamine salt) with VaporGrip technology were developed to reduce dicamba volatility and drift; however, there are claims that these products can still volatilize or drift. Field studies were conducted to evaluate glyphosate (N-(phosphonomethyl)glycine)–resistant (GR) soybean response to micro-rates (0, 1/1000, 1/500, 1/100, 1/50, and 1/10 of the label rate, 560 g a.e. ha⁻¹) of the two new dicamba products compared with Clarity (diglycolamine salt) applied at three growth stages. The GR soybean [Glycine max (L.) Merr.] was equally impacted by the micro-rates of all three products as measured by visual injury, height reduction, delayed physiological maturity, and yield reduction. The greatest visual injury (80%), plant height reduction (65%), maturity delay (22 d), and soybean yield loss (96%) was caused by 1/10 of the dicamba label rate when applied at V7/R1 soybean growth stage. In addition, estimation of effective dose for 5, 10, or 20% yield reduction suggested that V7/R1 was the most sensitive soybean growth stage to the three dicamba products. For example, 10% yield reduction occurred when 1.83 to 1.85 g a.e. ha⁻¹ (~1/300 of the label rate) of Engenia was applied at V2 or R2, whereas, a lower dose of 0.32 g a.e. ha⁻¹ (1/1750 of the label rate) of Engenia caused the same level of yield reduction when applied at V7/R1. Similar doses were estimated for Clarity and XtendiMax; therefore dicamba drift should be avoided at all costs, because GR soybean was equally sensitive to low rates of all three tested products with different formulations or technologies.

Dicamba (3,6-dichloro-2-methoxybenzoic acid) use is on the increase, especially in dicamba-resistant (DR) soybean [Glycine max (L.) Merr.] and cotton (Gossypium hirsutum L.) for control of broadleaf weeds that are hard-to-control and/or resistant to other herbicides (Norsworthy et al., 2011; Meyer et al., 2015; Ganie and Jhala, 2017; Osipitan and Dille, 2017; Underwood et al., 2017). However, the off-target movement of dicamba-based herbicides to non-DR soybean and other broadleaf crops has become a major concern. During the 2017 growing season, 1.2 million ha of crops were injured by dicamba drift, which led to various forms of litigations (Bradley, 2017a; Knezevic et al., 2018; Secor, 2018). The negative impact of dicamba on non-DR soybean can vary with soybean type, dicamba rate, and soybean growth stage at the time of drift occurrence. Drift is possible at different growth stages of sensitive soybean, because in DR crops application can be made at pre-plant, at planting, and post-emergence (up to flowering time) (Griffin et al., 2013).

The majority of the 36 million ha of soybean in the United States are planted to non-DR varieties such as glyphosate (N-(phosphonomethyl)glycine)–resistant (GR) soybean (Bradley, 2017b; Secor, 2018). The off-target movement of dicamba from surrounding fields is a potential threat to GR soybean production. Dicamba injury in GR soybean can cause reduction in growth, development and grain yield (Robinson et al., 2013; Solomon and Bradley, 2014; Soltani et al., 2016). The injury symptoms caused by dicamba on...
sensitive soybean include cupping of leaves, strapping, epinasty, stunted growth, curling of pods, and necrosis (Johnson et al., 2010; Griffin et al., 2013; McCown, 2018).

Off-target movement of dicamba from physical drift during the application is a common risk with dicamba use. However, further movement following the application from fine aerosol droplets that remain suspended during air temperature inversions, high air relative humidity, and low wind speeds can also occur (Behrens and Lueschen, 1979; Strachan et al., 2010). These suspended tiny aerosol-size dicamba droplets may not evaporate for some time, and thus can drift from the target site, especially in the first 36 h after application (Bradley, 2017b; Mueller, 2017).

Studies have shown that dicamba formulations such as dicamba acid, dimethylamine salt (Banvel), and diglycolamine salt (Clarity) are volatile, could drift, and cause various degree of injury and yield loss ranging from 3 to 70% (Andersen et al., 2004; Johnson et al., 2010; Griffin et al., 2013; Robinson et al., 2013). New dicamba-based herbicides such as Engenia (N,N-bis-(3-aminopropyl) methylamine salt) and XtendiMax (diglycolamine salt) with VaporGrip technology were developed with the primary purpose of reducing dicamba volatility and drift. Reports have shown that the new dicamba products can still move off-target (Hartzler, 2017). It is unclear if a drift from these dicamba products has differential impact on GR soybean. Our objective was, therefore, to evaluate the response of GR soybean to micro-rates of the two new dicamba herbicides (Engenia and XtendiMax) and as well as Clarity applied at three soybean growth stages.

**MATERIALS AND METHODS**

**Experimental Site**

A total of three field studies were conducted at Haskell Agricultural Laboratory of the University of Nebraska-Lincoln in Concord, NE (42.37°N, 96.68°W), of which one dryland study in 2016 and two studies (dryland and irrigated) in 2017. The two study sites in 2017 were about 1.2 km apart. Glyphosate-resistant soybean, Pioneer P24T19R was seeded at 370,120 seeds ha⁻¹ within the first week of June in both years and sites. Each experimental plot had four rows of the GR soybean with a dimension of 8 by 3 m. The tillage practice of the fields was no-till previously cultivated with corn (Zea mays L.). The total rainfall from May to September were 416 and 352 mm in 2016 and 2017, respectively, compared with a 30-yr average of 450 mm (Table 1). The average daily air temperature from May to September were 16 and 19°C in 2016 and 2017 respectively, compared with 20°C (30-yr average, Table 1). The irrigated sites received additional 203 mm of water between May and September in 2017. The total amount of water (rainfall only) within 1 wk after herbicide applications was 17 to 35 mm at the dryland (2016 and 2017) depending on application date, whereas the irrigated site received a total of 46 to 53 mm of water (rainfall plus irrigation) within the same period.

**Experimental Design**

The studies were arranged in a split-split-plot design with four replications. The main plot treatments were three application times/stages including second trifoliate (V2), seventh trifoliate/ beginning of flowering (V7/R1), and full flowering (R2) soybean growth stage. The date difference between the V7/R1 and R2 stage was approximately 8 d. The sub-plot treatments were three dicamba herbicides: Clarity (dicamba diglycolamine salt, 480 g L⁻¹; BASF Corporation, 26 Davis drive, Research Triangle Park, NC), Engenia (dicamba N,N-bis-(3-aminopropyl) methylamine salt, 600 g L⁻¹; BASF Corporation, Research Triangle Park, NC), and XtendiMax (dicamba diglycolamine salt, 350 g L⁻¹; 800 North Lindbergh Blvd, St. Louis, MO). The sub-sub plot treatments were 6 micro-rates of dicamba (0, 1/1000, 1/500, 1/100, 1/50, and 1/10 of the label rate; 560 g a.e. ha⁻¹). Dicamba applications were made using a CO₂– pressurized backpack boom sprayer calibrated to deliver 140 L ha⁻¹ at 276 kPa through four 11004-VP flat spray nozzles (Turbo TeeJet Induction, Spraying Systems Co., Wheaton, IL). Wind speeds at the time of treatment applications were between 6 and 9 km h⁻¹ and the air temperatures were 18 to 20°C (2016), 21 to 25°C (2017, dryland site), and 24 to 29°C (2017, irrigated site).

**Data Collection and Analysis**

Visually rated soybean injuries on the scale of 0 (no injury) to 100 (dead plant) were collected at 7, 14, 21, and 28 days after treatment (DAT). The injury symptoms included cupping of leaves, leaf and stem epinasty, stunting, swollen stem, curly pod, and necrosis, depending on the dicamba rate and growth stage of application. The cumulative severity of these symptoms were used for injury rating, relative to each growth stage of application. Plant height of five randomly selected soybean plants was collected at 7, 14, 21, and 28 DAT. Number of branches, days to canopy closure, number of flowering nodes, and days to physiological maturity of soybean plants was also documented. Combine (Almaco SP40, Nevada, IA) was used to harvest 8 m of two middle rows in each plot in October of each year, with yields reported at 13% moisture.

There were significant interactions between treatments and site of study (dryland vs. irrigated) on grain yield; therefore, the yield data were presented separately for dryland and irrigated. A four-parameter log-logistic regression model was used to analyze the relationship between dicamba micro-rates, and various parameters (visual injury, plant height, days to physiological maturity, grain yield, or yield components) (Knezevic et al., 2007). The regression analyses helped estimate the dicamba doses (ED values) causing a range of injury levels (e.g., 10, 20, and 50% threshold), height reduction, maturity delay, and yield losses (Knezevic et al., 2007). The estimated dicamba doses (ED values) were used to determine the sensitivity of the GR soybean to the dicamba products at different growth stages; the lower the required dose, the higher the sensitivity.
Table 2. Dose of dicamba products that resulted in 10% (ED_{10}), 20% (ED_{20}), and 50% (ED_{50}) injury of glyphosate-resistant soybean applied at three growth stages.

<table>
<thead>
<tr>
<th>Dicamba</th>
<th>Growth stage</th>
<th>ED_{10} (SE)†</th>
<th>ED_{20} (SE)</th>
<th>ED_{50} (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clarity</td>
<td>V2</td>
<td>0.01 (0.01)</td>
<td>0.04 (0.02)</td>
<td>0.72 (0.07)</td>
</tr>
<tr>
<td></td>
<td>V7/R1</td>
<td>0.03 (0.01)</td>
<td>0.08 (0.01)</td>
<td>0.45 (0.09)</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>0.07 (0.00)</td>
<td>0.49 (0.01)</td>
<td>1.78 (0.81)</td>
</tr>
<tr>
<td>Engenia</td>
<td>V2</td>
<td>0.22 (0.11)</td>
<td>0.36 (0.11)</td>
<td>0.99 (0.18)</td>
</tr>
<tr>
<td></td>
<td>V7/R1</td>
<td>0.02 (0.00)</td>
<td>0.06 (0.01)</td>
<td>0.40 (0.08)</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>0.01 (0.00)</td>
<td>0.06 (0.01)</td>
<td>1.08 (0.14)</td>
</tr>
<tr>
<td>XtendiMax</td>
<td>V2</td>
<td>0.09 (0.04)</td>
<td>0.19 (0.06)</td>
<td>0.71 (0.05)</td>
</tr>
<tr>
<td></td>
<td>V7/R1</td>
<td>0.02 (0.00)</td>
<td>0.07 (0.01)</td>
<td>0.41 (0.04)</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>0.02 (0.00)</td>
<td>0.08 (0.01)</td>
<td>1.14 (0.15)</td>
</tr>
</tbody>
</table>

† Estimated doses (ED) were compared using standard errors (SE).

RESULTS AND DISCUSSION

There was no treatment × year × site interaction on all tested soybean response variables, except on grain yield. Thus, data were combined over years and sites (dryland and irrigated) for all response variables except grain yield. In addition, there were no significant differences among the dicamba products for all the soybean response variables (Fig. 1–5; Table 2–6). Results were generally presented in respect to the magnitude and sensitivity of soybean response variables to dicamba at different growth stages.

Visual Injury

Soybean visual injuries increased with increase in dicamba rates. The visual injuries ranged from 22 to 76% as dicamba rate increased from 1/1000 to 1/10 of the label rate (560 g a.e. ha\(^{-1}\)) at 21 DAT; averaged across all products and growth stages of application (Fig. 1). Injury symptoms and severity largely depended on the soybean growth stage at the time of dicamba application. Application of dicamba at the V2 growth stage caused injury ranging from 29 to 75% at 21 DAT as dicamba rate increased from 1/1000 to 1/10 of label rate averaged across dicamba products. Similarly, Soltani et al. (2016) reported 22 to 79% soybean visible injury with dicamba rate of 1/747 to 1/10 of the label rate when applied at the V2 growth stage. Also, Andersen et al. (2004) reported as much as 80% visual injury with 1/10 of dicamba label rate.

In addition, soybean injury was generally higher in soybean treated with dicamba at V7/R1 compared with V2 and R2 growth stages. For example, when dicamba was applied at V7/R1 with 1/10 rate, injury levels were around 80% compared with 59% when dicamba was applied at the R2 stage. Study by Robinson et al. (2013) also showed lower injury level at the R2 stage compared with vegetative stages (V2 and V5).

The sensitivity of each growth stage to Engenia and XtendiMax as measured by visual injury was not different from that of Clarity (Table 2). The required dose of each of the dicamba-based herbicides for 50% injury was generally lowest at the V7/R1 stage, indicating that the beginning of flowering was the most sensitive stage to the dicamba herbicides (Table 2). For example, 0.40 g a.e. ha\(^{-1}\) of Engenia applied at the V7/R1 stage caused 50% injury compared with higher rates at V2 (0.99 g a.e. ha\(^{-1}\)) and R2 (1.08 g a.e. ha\(^{-1}\)) stages. Similarly, estimated dose of XtendiMax that caused 50% injury was 0.41, 0.71, and 1.14 g a.e. ha\(^{-1}\) for the V7/R1, V2, and R2 growth stages, respectively. The estimated doses of Engenia and XtendiMax were generally similar to those of Clarity for all growth stages. Robinson et al. (2013) reported similar results when Clarity was applied at the late vegetative stage (V5) than at the early vegetative (V2) and full flowering (R2) stages.

Plant Height Reduction

Reduction in the GR soybean plant height caused by the dicamba products increased with increase in dicamba rates from 1/1000 to 1/10 of the label rate 28 DAT (Fig. 2). Height was reduced as much as 46, 65, and 21% at the V2, V7/R1, and R2 growth stages of application, respectively, by the highest rate (1/10 of label rate) across the dicamba products. Similarly, 62% reduction in soybean height was reported when dicamba was applied at 1/10 of label rate at R1 (Weidenhamer et al., 1989). All three products reduced soybean heights at about the same level when compared for the same growth stage of herbicide application (Table 3). Of the three growth stages, the V2 and R2 stages showed statistically similar reduction in plant height, whereas the V7/R1 stage was more sensitive to dicamba. For example, a dose range of 5.92 to 8.04 g a.e. ha\(^{-1}\) (equivalent to 1/95 to 1/70 of the label rate) applied at the V2 stage was required to cause 50% (~35 cm) reduction in plant height across all three products at 28 DAT. However, a significantly lower dose of 3.01 to 4.41 g a.e. ha\(^{-1}\) to 1/10 of label rate averaged across dicamba products. Similarly, Soltani et al. (2016) reported 22 to 79% soybean visible injury with dicamba rate of 1/747 to 1/10 of the label rate when applied at the V2 growth stage. Also, Andersen et al. (2004) reported as much as 80% visual injury with 1/10 of dicamba label rate.
Table 5. Parameter estimates and dose of dicamba products that resulted in 5% (ED$_{5}$), 10% (ED$_{10}$), and 20% (ED$_{20}$) yield reduction of glyphosate-resistant soybean sprayed at three growth stages at two study sites. Parameter B, C, D, and I$_{50}$ represent slope, lower limit, upper limit, and dose at inflection point, respectively.

<table>
<thead>
<tr>
<th>Dicamba</th>
<th>Growth stage</th>
<th>B (SE)†</th>
<th>C (SE)</th>
<th>D (SE)</th>
<th>I$_{50}$ (SE)</th>
<th>ED$_{5}$ (SE)</th>
<th>ED$_{10}$ (SE)</th>
<th>ED$_{20}$ (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clarity</td>
<td>V2</td>
<td>1.47 (0.77)</td>
<td>3033 (908)</td>
<td>4248 (105)</td>
<td>10.4 (2.04)</td>
<td>1.91 (0.43)</td>
<td>2.14 (0.72)</td>
<td>5.39 (1.24)</td>
</tr>
<tr>
<td></td>
<td>V7/R1</td>
<td>0.89 (0.12)</td>
<td>250 (50)</td>
<td>4250 (1001)</td>
<td>4.12 (2.07)</td>
<td>0.24 (0.12)</td>
<td>0.56 (0.21)</td>
<td>1.37 (0.37)</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>2.78 (1.6)</td>
<td>1800 (203)</td>
<td>4190 (702)</td>
<td>14.3 (3.50)</td>
<td>5.89 (2.32)</td>
<td>7.70 (2.67)</td>
<td>10.3 (2.43)</td>
</tr>
<tr>
<td>Engenia</td>
<td>V2</td>
<td>0.94 (0.20)</td>
<td>1600 (302)</td>
<td>3950 (201)</td>
<td>7.62 (2.06)</td>
<td>0.41 (0.18)</td>
<td>1.85 (0.32)</td>
<td>1.92 (0.76)</td>
</tr>
<tr>
<td></td>
<td>V7/R1</td>
<td>0.71 (0.11)</td>
<td>150 (26)</td>
<td>4171 (184)</td>
<td>3.01 (1.06)</td>
<td>0.12 (0.06)</td>
<td>0.32 (0.13)</td>
<td>0.91 (0.26)</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>1.89 (0.42)</td>
<td>1200 (269)</td>
<td>4020 (401)</td>
<td>7.21 (2.02)</td>
<td>0.87 (0.21)</td>
<td>1.83 (0.15)</td>
<td>4.12 (1.02)</td>
</tr>
<tr>
<td>XtendiMax</td>
<td>V2</td>
<td>1.20 (0.14)</td>
<td>2200 (359)</td>
<td>4246 (157)</td>
<td>8.41 (1.04)</td>
<td>0.45 (0.15)</td>
<td>1.95 (0.33)</td>
<td>2.14 (0.74)</td>
</tr>
<tr>
<td></td>
<td>V7/R1</td>
<td>0.97 (0.15)</td>
<td>300 (56)</td>
<td>4380 (124)</td>
<td>3.41 (1.44)</td>
<td>0.29 (0.15)</td>
<td>0.63 (0.26)</td>
<td>1.48 (0.44)</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>1.20 (0.12)</td>
<td>1750 (101)</td>
<td>4447 (205)</td>
<td>8.00 (2.19)</td>
<td>1.05 (0.21)</td>
<td>2.22 (0.53)</td>
<td>5.00 (1.19)</td>
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</table>
|† Means among rates and between growth stages were compared using standard errors (SE).

† Estimates were compared using standard errors (SE).
was needed for 50% height reduction for the V7/R1 stage across all three products 28 DA T (Table 3). Previous studies also showed that early flowering (R1) stage was the most sensitive to dicamba (Auch and Arnold 1978; Solomon and Bradley 2014). The reduction in plant height caused by the dicamba products was followed by lateral branching when applied at the V2 growth stage with the rate of 1/100 to 1/10 of the label rate (data not shown). The lateral branching was caused by the loss of apical dominance, which then promoted the release of lower leaf axillary buds (Andersen et al., 2004).

In addition, reduction in plant height contributed also to a significant delay in canopy closure when the dicamba micro-rates were applied at V2 and V7/R1 stages. For example, a V2 stage application delayed canopy closure by 7 to 20 d as the rates increased from 1/100 to 1/10 of the label rate across all three products (Table 3). The delay was further increased by 18 to 26 d when 1/100 to 1/10 of the label rate of dicamba was applied at the V7/R1 stage regardless of the product. Generally, synthetic auxin herbicides are known to cause reduced plant growth, in particular the apical meristem, leaves, and petioles in sensitive plants.

### Delay in Physiological Maturity

All three products similarly delayed physiological maturity (Table 3), especially as dicamba rates increased from 1/1000 to 1/10 of the label rate (560 g a.e. ha$^{-1}$) (Fig. 3). The highest rate of dicamba (1/10 of the label rate) caused 16, 22, and 20 d delay in

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### Table 6. Dicamba dose that caused 5% (ED$_{5}$) and 10% (ED$_{10}$) reduction in pods plant$^{-1}$, seeds pod$^{-1}$, and 100-seed weight reduction of glyphosate-resistant soybean sprayed at three growth stages.

<table>
<thead>
<tr>
<th>Dicamba</th>
<th>Growth stage</th>
<th>Pods plant$^{-1}$</th>
<th>Seeds pod$^{-1}$</th>
<th>100-seed weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ED$_{5}$ (SE)$^\dagger$</td>
<td>ED$_{10}$ (SE)</td>
<td>ED$_{5}$ (SE)</td>
</tr>
<tr>
<td>Clarity</td>
<td>V2</td>
<td>2.19 (1.91)</td>
<td>9.05 (3.22)</td>
<td>5.02 (0.26)</td>
</tr>
<tr>
<td></td>
<td>V7/R1</td>
<td>1.34 (1.17)</td>
<td>3.71 (1.74)</td>
<td>1.92 (0.01)</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>2.28 (1.13)</td>
<td>7.17 (1.42)</td>
<td>4.69 (0.29)</td>
</tr>
<tr>
<td>Engenia</td>
<td>V2</td>
<td>1.76 (0.20)</td>
<td>6.95 (1.24)</td>
<td>4.48 (0.19)</td>
</tr>
<tr>
<td></td>
<td>V7/R1</td>
<td>0.77 (0.29)</td>
<td>2.21 (0.83)</td>
<td>1.09 (0.13)</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>1.57 (0.90)</td>
<td>5.89 (2.05)</td>
<td>4.44 (0.27)</td>
</tr>
<tr>
<td>XtendiMax</td>
<td>V2</td>
<td>1.73 (0.02)</td>
<td>7.33 (1.83)</td>
<td>5.12 (0.06)</td>
</tr>
<tr>
<td></td>
<td>V7/R1</td>
<td>0.75 (1.20)</td>
<td>2.31 (0.74)</td>
<td>2.02 (1.02)</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>1.98 (0.36)</td>
<td>6.98 (2.27)</td>
<td>5.42 (0.41)</td>
</tr>
</tbody>
</table>

$^\dagger$ Estimates were compared using standard errors (SE).
physiological maturity when applied at V2, V7/R1, and R2 growth stages, respectively, across all dicamba products (Fig. 3).

Furthermore, the time of physiological maturity was the most delayed when applied at the V7/R1 stage (Table 3). For example, a significantly lower dicamba dose of 0.04 g a.e. ha\(^{-1}\) applied at the V7/R1 stage compared with higher doses (0.39 or 0.41 g a.e. ha\(^{-1}\) applied at V2 and R2 stages, respectively) was required to cause 10% (~3 d) delay in physiological maturity averaged across all products. A previous report has shown greater delay in soybean maturity following dicamba applied at early reproductive stage compared with vegetative stages (Wax et al., 1969).

**Soybean Yields and Yield Components**

Yields of the non-treated GR soybean were statistically similar within each site (Fig. 3; Table 5). The average yields of non-treated soybean at the dryland and irrigated site were 4211 and 4440 kg ha\(^{-1}\), respectively. Application of any of the three dicamba products significantly affected the GR soybean yield with greater impact at the dryland compared with irrigated site. Of all stages, the V7/R1 growth stage appeared the most affected. For example, at the dryland, soybean applied with 1/100 or 1/10 of XtendiMax label rate at the V2 stage yielded 3100 to 2200 kg ha\(^{-1}\). The same rates of XtendiMax when applied at the R2 stage lowered yield to 3200 to 1750 kg ha\(^{-1}\). A much lower yield of 1700 and 210 kg ha\(^{-1}\) was obtained when the same rates (1/100 or 1/10) of XtendiMax was applied at the V7/R1 soybean stage, suggesting that the V7/R1 was the most sensitive stage. Similar results were obtained with application of Engenia and Clarity (Fig. 3, Table 5).

The impact of the three dicamba products on yield was significantly lower in irrigated soybean. For example, irrigated soybean sprayed with 1/100 or 1/10 of XtendiMax label rate at the V2 stage yielded 3700 and 3200 kg ha\(^{-1}\), respectively, compared with significantly lower yields (3100 and 2200 kg ha\(^{-1}\)) in dryland soybean. Similar yields were obtained with application of Engenia and Clarity (Fig. 3, Table 5).

The yield components, including pod plant\(^{-1}\), seeds pod\(^{-1}\), and 100-seed weight were influenced by the rates of the dicamba products and growth stage of dicamba application (Fig. 4, Table 6). Soybean in non-sprayed plots yielded about 28 pods plant\(^{-1}\), 3 seeds pod\(^{-1}\), and 15 g of 100-seed weight. However, the yield components were significantly reduced by all rates of dicamba products and the application time (Fig. 4). For example, number of pods per plant was reduced from 28 down to 15 pods plant\(^{-1}\) in the V2 stage and only 5 pods plant\(^{-1}\) in plots sprayed at the V7/R1 stage, averaged across all

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Fig. 4. Soybean yield components as influenced by increasing micro-rates of dicamba products at different growth stages of application. Data was combined for both sites and years.
three dicamba products, suggesting again that the V7/R1 stage was the most sensitive stage. Similarly, the V7/R1 stage was most sensitive stage for reduction in seeds per pod and 100-seed weight (Table 6). For example, Engenia doses of 2.22, 6.02, and 6.16 g a.e. ha$^{-1}$ caused 10% reduction in seeds per pod when applied at V7/R1, R2, and V2 stages, respectively. Similar results were observed with Clarity and XtendiMax (Table 6). Seed weight was reduced 10% with 1.97, 4.03, and 4.13 g a.e. ha$^{-1}$ of Engenia when applied at V7/R1, V2, and R2 stages, respectively, and similar results were obtained with the use of other products (Clarity and XtendiMax). Previous studies also reported negative impact of synthetic auxin herbicides (including dicamba) on soybean seed components such as pods per plant, seeds per pod, seed weight, and reproductive nodes (Kelley et al., 2005; Robinson et al., 2013; Wax et al., 1969).

**Soybean Yield Reduction**

Reduction in the GR soybean yield caused by the dicamba products increased with increase in dicamba rates from 1/1000 to 1/10 of the label rate (Fig. 5). The highest dicamba rate (1/10 of the label rate) reduced soybean yield by as much as 96% (Fig. 5). However, there was significant difference in yield loss between the irrigated and dryland site.

All three products equally reduced the GR soybean yields (Fig. 5, Table 5). For example, Clarity treated soybean at the V2 stage with 1/10 of the label rate resulted in 47% (1945 kg ha$^{-1}$) yield reduction, which was not significantly different from 51% (2146 kg ha$^{-1}$) and 48% (2046 kg ha$^{-1}$) yield reduction in Engenia and XtendiMax treated soybean, respectively (Fig. 3, Table 5). Of the three stages, the yields of GR soybean treated with the dicamba products at the V7/R1 stage was the most reduced. For example, Clarity treated soybean at the V7/R1 stage with 1/10 of the label rate resulted in 96% (4000 kg ha$^{-1}$) yield reduction, which was similar to 95% (4021 kg ha$^{-1}$) and 94% (3994 kg ha$^{-1}$) yield reduction in Engenia and XtendiMax treated soybean, respectively. The observed pattern of yield reduction is consistent with previous reports that evaluated impact of dicamba on soybean yield at different growth stages (Auch and Arnold, 1978; Kelley et al., 2005; Robinson et al., 2013).

Similar to the dryland experimental site, all three products equally reduced the GR soybean yields (Fig. 5, Table 5). However, there was less reduction in yield at the irrigated site compared with dryland for soybean treated with dicamba at the V2 and R2 stages (Fig. 5). For example, at the irrigated site, 1/10 of dicamba label rate applied at the V2 stage resulted in 34% (1606 kg ha$^{-1}$) yield reduction compared with significantly greater yield reduction of 49% (2046 kg ha$^{-1}$) at the dryland site, averaged across the three dicamba products. Similarly, GR soybean treated with 1/10 of dicamba label rate at the R2 stage resulted in an average of 46% (2180 kg ha$^{-1}$) yield reduction compared with significantly greater (57%) yield reduction at the dryland, across dicamba products.

Furthermore, the estimated effective doses (ED) showed that the yields of GR soybean at the dryland experimental site compared with the irrigated site were generally more sensitive to the dicamba rates, and V7/R1 was the most sensitive growth stage irrespective of the site of study. At the dryland, estimated Engenia dose of 1.85 g a.e. ha$^{-1}$ caused 10% yield reduction when applied at the V2 growth stage, but at the V7/R1 stage of application, Engenia dose of 0.32 g a.e. ha$^{-1}$ caused the same level of yield reduction. At the irrigated site, estimated Engenia dose of 6.14 g a.e. ha$^{-1}$ caused 10% yield reduction when applied at the V2 growth stage, whereas at the V7/R1 stage of application, Engenia dose of 2.17 g a.e. ha$^{-1}$ caused the same level of yield reduction. Similar doses were estimated for Clarity and XtendiMax at each site of the studies.

Similarly, Weidenhamer et al. (1989) reported that 10% yield reduction was caused by 0.4 g a.e. ha$^{-1}$ of dicamba in a dryland location compared with higher amount (1.5 g a.e. ha$^{-1}$) of dicamba required in a location with adequate water. These results suggested that the sensitivity of soybean yield to the dicamba products could be influenced by amount of water the soybean plant received during the growing season. For example, in this current study, the total rainfalls (229 mm in 2016 and 173 mm in 2017) during the early part of the growing season (May–July) were below the 30-yr average (291 mm) at both irrigated and non-irrigated sites. Out of these rainfalls, a total rainfall within 1 wk after dicamba application was 21 mm (in 2016) and 17 mm (in 2017) for V2 application timing at both irrigated and dryland sites, respectively. Therefore, we hypothesize that an increased amount of water—46 mm (4 mm of rainfall plus 42 mm from irrigation)—within 5 d after dicamba application at the irrigated site (in 2017)—could have promoted a quicker detoxification of low rates of dicamba, and allow early recovery from injury in plants, as previously suggested by Dexter and Slife (1971) and Robinson et al. (2013).

Overall, there was no difference in the estimated dose of dicamba required to cause 10% yield loss when applied at V2 and R2 soybean growth stages. Previous studies also suggested that soybean yield was more sensitive to dicamba applied at early reproductive stage (R1) compared with early vegetative stages (Auch and Arnold, 1978; Griffin et al., 2013; Soltani et al., 2016). There was lack of information in the past on comparative sensitivity of grain yield
between soybean treated with dicamba at early flowering (V7/R1) and full flowering (R2) stages. Our study clearly showed that soybean is more sensitive to dicamba applied at early flowering stage than at full flowering stage. Flower abortion caused by the highest tested dicamba rate was six times higher when applied at the early flowering stage than at the full flowering stage (data not shown).

**Agronomic Implications**

All three dicamba products (Clarity, Engenia, and XtendiMax) had statistically similar effects on the growth and yield of the sensitive soybean. Injury symptoms and severity largely depended on the growth stage of dicamba application, with application just before flowering resulting in the greatest soybean injury. Reduction in plant height was a good early indicator of severity of dicamba injury. In addition, reduction in soybean height resulted in delayed canopy closure even with the lowest tested dicamba rate (1/1000 of label rate). Reduction in crop height coupled with delayed canopy closure could imply reduction of soybean competitive ability against weeds, which can further result in potentially higher yield losses than reported in this article. Furthermore, delayed soybean maturity can also delay harvest time, which can make soybean subject to early frost.

Our results suggested that soybean yield loss caused by the dicamba micro-rates could be explained by response in the yield components. Robinson et al. (2013) reported that each yield component had varied degree of influence on the final seed yield. For example, their results suggested that impact of pods per m² on soybean yield was four times greater than that of seeds per pod on yield. Soybean yield was the most sensitive to the dicamba products applied at the V7/R1 growth stage compared with the R2 and V2 stages resulting from disruption of flowering stage (lack of flower production and/or abortion of flowers) and impaired yield components. The severity of yield reduction may also depend on the amount of rainfall or irrigated water the soybean plant received after the application of dicamba. In conclusion, all tested rates of dicamba caused considerable degree of injury, growth damage, and yield loss in the GR soybean; therefore, efforts must be made to avoid drift of dicamba onto sensitive soybean, irrespective of the dicamba product technology or formulation.

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