

Dicamba-Tolerant Soybean Response to 2,4-D Micro-Rates at Three Growth Stages

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ABSTRACT

While dicamba and 2,4-D belong to the same herbicide group, dicamba- and 2,4-D-tolerant soybeans are not cross-resistant. A study was conducted to investigate dicamba-tolerant (DT) soybean response to 2,4-D micro-rates at three growth stages. The study used a randomized complete block design with six replications and a split-plot treatment arrangement. Main plots consisted of three 2,4-D application times [second trifoliate (V2); beginning of flowering (R1); and full flowering (R2)] and subplots consisted of six 2,4-D micro-rates (1/5; 1/10; 1/50; 1/100; 1/500; and 1/1,000 of the label recommended dose of 1,120 g ae ha⁻¹) and a check with no herbicide applied. DT soybean injury symptoms were assessed at 7, 14 and 21 days after treatment (DAT). Yield components, grain yield and yield loss were estimated. The greatest soybean injury (59%) at 21 DAT, delay in canopy closure (16.8 d), and reduction in number of branches plant⁻¹ (52%), pods plant⁻¹ (38%) and 100 seed weight (16%) which ultimately resulted in yield loss (78%) was caused by 1/5 of the label recommended dose of 2,4-D (214 g ae ha⁻¹) at R1 growth stage. Based on effective dose (ED) estimates, 8.66 g ae ha⁻¹ 2,4-D caused 0.22 Mg ha⁻¹ (5%) yield loss in DT soybean at R1 but a 1.34- and 2.11-fold higher dose of the same herbicide was required to cause equal yield loss at R2 and V2 growth stages, respectively. Similarly, the number of branches plant⁻¹, pods plant⁻¹ and flowers plant⁻¹ were more sensitive to 2,4-D at R1 than V2 and R2 growth stages with the latter being the most sensitive yield component. On the other hand, 100 seed weight was 3.1- and 4.5-fold more sensitive to 2,4-D at R2 than R1 and V2 growth stages, respectively. Overall, these results showed that DT soybean is most sensitivity to 2,4-D at R1. Nomenclature: Soybean, *Glycine max* (L.) Merr.

Keywords: Dicamba-tolerant soybean, 2,4-D micro-rates, growth stage.

INTRODUCTION

Herbicides are essential for weed control; however, finding new herbicides and or new herbicide modes of action has been quite a challenge (Duke 2012). To address this issue, herbicide-resistant crops have been developed and widely promoted especially in the U.S. (Dekker and Duke 1995; Mazur and Falco 1989; Owen et al. 2005; USDA 2020) where herbicide

resistant weeds are a major threat to crop production causing massive yield losses that account for billions of dollars annually (Inderjit 2009; Oerke 2006). Synthetic auxin herbicides (e.g. 2,4-D and dicamba) were first discovered in the first half of the twentieth century and have been used primarily for broadleaf weed control in monocot crops (Egan et al. 2014; Peterson et al. 2016). However, it wasn't until the recent launching of synthetic auxin-resistant crops (e.g. cotton and soybean) that the use of synthetic auxin herbicides in dicot crops increased dramatically particularly in the U.S. (USDA 2020).

Soybean [*Glycine max* (L.) Merr.] is an important crop in the U.S. and a major contributor to the country's agriculture sector's value (USDA 2020). According to the USDA (2020), majority of soybean hectares in the U.S. are planted to cultivars with herbicide-resistance traits including the Roundup Ready® Xtend Crop System (Bayer Co., St. Louis, Mo, 63167) and the Enlist® Weed Control System (Corteva Agriscience, Indianapolis, IN, 46268). The former has been genetically modified with transgenes that confer resistance to glyphosate and dicamba and the latter has been genetically modified with transgenes that express resistance to glyphosate, 2,4-D and glufosinate. Specifically, resistance to dicamba and 2,4-D is conferred by dicamba monooxygenase bacterial transgene in Roundup Ready Xtend soybean (Behrens et al. 2007) and aryloxyalkanoate dioxygenase bacterial transgene in Enlist E3 soybean (Wright et al. 2010), respectively. These modifications allow in-season application of dicamba and 2,4-D over soybean. Dicamba and 2,4-D have the same mode of action but are not cross-resistant; dicamba is a benzoic acid and 2,4-D is a phenoxy acid herbicide (Grossmann 2010). In addition, both dicamba and 2,4-D are prone to volatilization thus can move off-target and damage susceptible crops. To address this problem, low-volatile dicamba formulations have been developed for use on Roundup Ready Xtend soybean including Xtendimax™, Fexapan™, and Engenia™. Similarly, low-volatile 2,4-D formulations such as Enlist One and Enlist Duo have been developed for use on Enlist E3 soybean. However, dicamba-resistant soybean is sensitive to 2,4-D, and 2,4-D-resistant soybean is sensitive to dicamba. Apart from weed control, the high risk of injuring soybean not carrying one of the traits is what drives adoption of these technologies. This is why the use of synthetic auxin herbicides has generated some debate in recent years. More debate is expected, however, as the new Enlist E3 technology becomes more popular among soybean growers.

Earlier studies have mainly focused on the effects of dicamba-based herbicides on dicamba-sensitive soybean (Osipitan et al. 2019). However, with the recent launching of Enlist E3 soybean, in-season application of 2,4-D on soybean is expected to increase which is likely to favor occurrence of drift and tank contamination with the herbicide. Therefore, more data are needed on the impact of 2,4-D on non 2,4-D-resistant soybean. In addition, more research is required to further our understanding of how timing of occurrence of drift and or tank contamination with 2,4-D influences the impact of the herbicide on 2,4-D-sensitive soybean. The objective of this study was to investigate dicamba-tolerant (DT) soybean response to 2,4-D micro-rates applied at three growth stages.

MATERIALS AND METHODS

Experimental Site

This study was conducted under irrigation at two locations in eastern Nebraska. In 2019, the location was at the University of Nebraska-Lincoln Haskell Agricultural Laboratory near

Concord, NE (HAL; 42.388, -96.957, 450 m); and, in 2020, the location was at the University of Nebraska-Lincoln Eastern Nebraska Research and Extension Center near Mead, NE (ENREC; 41.151, -96.447; 355 m). HAL had Nora silty clay loam, 6 to 11% slope (fine-silty, mixed, mesic Udic Haplustolls) with mean precipitation of 696 mm yr⁻¹; and ENREC had Yutan silty clay loam, 2 to 6% slope (fine-silty, mixed, superactive, mesic Mollic Hapludalfs) with mean precipitation of 748 mm yr⁻¹.

Experimental Procedure

The study utilized a randomized complete block design with a split plot arrangement of treatments and 6 replications. The main plot treatments consisted of 3 application times and the subplot treatments consisted of 7 micro-rates of 2,4-D (Enlist One; Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN). The application times were: V2 (2nd trifoliolate), R1 (beginning of flowering) and R2 (full flower); and the 2,4-D micro-rates were 0; 1/5; 1/10; 1/50; 1/100; 1/500; and 1/1,000 of the label recommended rate (1,120 g ae ha⁻¹). Each subplot was 7.6 m long with four rows of DT soybean spaced 0.76 m apart. The V2 timing simulated occurrence of drift or tank contamination with 2,4-D at an early soybean growth stage, which represents the earliest Enlist One application scenario. The 2nd and 3rd timings simulated drift or tank contamination with 2,4-D at the later soybean growth stages that might be expected in other scenarios (eg. late plantings) where the herbicide is likely to be applied later around flowering time. The study was kept weed-free throughout the two growing seasons by hand weeding as needed. All herbicide treatments were applied using a CO₂-pressurized backpack boom sprayer. Wind speed at the time of herbicide application was 6-9 km h⁻¹ and air temperature was 20-22 °C in 2019 and 21-24 °C in 2020.

Data Collection and Analysis

Soybean injury symptoms were assessed on a scale of 0 (no injury) to 100 % (complete death) at 7, 14 and 21 days after treatment (DAT). Soybean was harvested with a small-plot combine. Number of branches plant⁻¹ and pods plant⁻¹ as well as 100 seed weight (g) were collected. In addition, yield and yield loss were estimated. Interaction effects between year, growth stage and 2,4-D micro rates on soybean yield and yield components were tested for significance at $\alpha = 0.05$. If the year was not significant, data were pooled before subjecting to dose response analysis using the drc package in R version 3.4.1 (R Core Team 2017). Visual estimates of DT soybean injury symptoms and yield (including yield components and loss) were regressed over 2,4-D micro-rates using a four-parameter log-logistic equation (Knezevic et al. 2007). The regression analysis were used to estimate the 2,4-D doses (ED values) causing 5, 10 and 20% injury, reduction in yield and yield components, and or loss at different growth stages. Standard errors were also estimated.

RESULTS AND DISCUSSION

Year by treatment interaction effect was not significant; therefore, the two year data were combined.

Soybean Injury Symptoms

Soybean injury symptoms consisted of bubbling and strapping of leaves and twisting of branches and stems which ultimately reduced plant height (data not shown). Epinasty was more evident when 2,4-D micro-rates were applied at V2 than R1 and R2 growth stages;

however, plants exposed to 2,4-D at the vegetative growth stage did eventually outgrow injury symptoms caused by the lowest micro-rates of the herbicide (data not shown). Similar injury symptoms have been reported elsewhere (Robinson et al. 2013; Egan et al. 2014). In general, soybean injury symptoms only became evident (noticeable) when 2,4-D was applied at 1/50 (V2 and R2) and 1/100 (R1) of the label recommended rate. Soybean injury symptoms increased with increase in 2,4-D micro-rates with the degree of injury varying depending on herbicide application timing, Figure 1A. R1 was the most sensitive growth stage. Increase in 2,4-D micro-rate from 1/1,000 to 1/5 of the label recommended rate caused soybean injury symptoms ranging from 0 to 45% at V2, 0 to 59% at R1 and 0 to 39% at R2 growth stage, respectively at 21 DAT suggesting that DT soybean is more sensitive to 2,4-D at R1 than V2 and R2 growth stages as also evidenced by the ED value estimates in Table 1. Based on the ED value estimates (Table 1), 2,4-D doses of 25.9, 24.0 and 27.7 g ae ha⁻¹ were required to cause 5% injury symptoms to DT soybean at V2, R1, and R2 growth stages at 21 DAT, respectively. This suggests that DT soybean is 1.1- and 1.2-fold more sensitive to 2,4-D at R1 than V2 and R2 growth stages, respectively.

Days to Canopy Closure

Increase in 2,4-D micro-rate delayed canopy closure with the number of days to canopy closure varying depending on timing of herbicide application. On average, DT soybean reached canopy closure in 64.9 d when left untreated. Conversely, DT soybean treated with 2,4-D had stunted and epinastic growth especially when the highest micro-rates of the herbicide were applied which ultimately delayed canopy closure irrespective of application timing, Figure 1B. For example, when DT soybean was treated with the lowest micro-rate of 2,4-D tested, 1/1,000 of the label recommended rate (1.07 g ae ha⁻¹) at V2, R1 and R2 growth stage, canopy closure was delayed by 1.1, 1.3 and 2.1 d on average, respectively. However, when DT soybean was treated with the highest micro-rate of 2,4-D tested, 1/5 of the label recommended rate (214 g ae ha⁻¹) at V2, R1 and R2 growth stage, canopy closure was further delayed by 8.3, 16.8 and 20.2 d on average, respectively. The increased delay in canopy closure with increased 2,4-D micro-rate is a result of reduced plant height (data not shown) due to stunted growth of DT soybean following herbicide application. Stunted growth has been reported to decrease plant leaf area (Costa et al. 2020) thus delaying canopy closure. Based on ED value estimates, a 2,4-D dose of 41.5, 13.1 and 10.9 g ae ha⁻¹ was required to delay DT soybean canopy closure by 5% (3.2 d) at V2, R1 and R2 growth stage, respectively (Table 1). This suggests that DT soybean is more sensitive to 2,4-D at reproductive stages with R1 and R2, respectively being 3.1- and 3.8-times more sensitive to the herbicide than V2 growth stage.

Number of branches plant⁻¹

Increase in 2,4-micro-rates reduced branching with the number of branches plant⁻¹ varying depending on plant growth stage at time of herbicide application. Prior to treatment, the number of branches plant⁻¹ were 2.8, 2.9 and 2.1 on average at V2, R1, and R2 growth stage, respectively. Following 2,4-D application, the number of branches plant⁻¹ was significantly reduced, Figure 1C. For example, when 2,4-D was applied at 1/1,000 of the label recommended rate (1.07 g ae ha⁻¹), the number of branches plant⁻¹ was reduced 34% to 1.9, 27% to 2.1 and 10% to 1.9 at V2, R1 and R2 growth stage, respectively. Similarly, when 2,4-D was applied at 1/5 of the label recommended rate (214 g ae ha⁻¹), the number of branches plant⁻¹ was reduced 65% to 1, 48% to 1.4 and 14% to 1.8 at V2, R1 and R2 growth stage, respectively. Based on ED

value estimates, a 2,4-D dose of 63.4, 13.9 and 146.5 g ae ha⁻¹ was required to reduce the number of branches plant⁻¹ in DT soybean by 5% (0.13) at V2, R1 and R2 growth stage, respectively (Table 1). This suggests that there is less or no reduction in the number of branches plant⁻¹ when DT soybean is exposed to 2,4-D at or past the full flowering stage.

Soybean Yield Components

Two soybean yield components that were significantly influenced by 2,4-D included the number of pods plant⁻¹ and 100 seed weight. The results are presented as follows:

Number of pods plant⁻¹

Generally, increase in 2,4-D micro-rate reduced DT soybean number of pods plant⁻¹ regardless of plant growth stage at time of herbicide application, Figure 2A. Of all growth stages, however, R1 appeared to be the most sensitive. For example, increase in 2,4-D micro-rate from 0 to 1/1,000 of the label recommended rate (1.07 g ae ha⁻¹) decreased DT soybean number of pods plant⁻¹ 5% from 53.4 to 50.8, 14% from 57.5 to 49.2, and 8% from 44.2 to 40.6 at V2, R1 and R2 growth stage, respectively. When 2,4-D micro-rate was increased from 0 to 1/5 of the label recommended rate (214 g ae ha⁻¹), DT soybean number of pods plant⁻¹ was decreased 22% from 53.4 to 41.4, 38% from 57.5 to 35.6, and 22% from 44.2 to 34.6 at V2, R1 and R2 growth stage, respectively. Based on the ED value estimates, a 2,4-D dose of 94.8, 39.2 and 46.5 g ae ha⁻¹ was required to reduce the number of pods plant⁻¹ by 5% (2.5) at V2, R1 and R2 growth stage, respectively, Table 2. This suggests that R1 is 2.4- and 1.2-fold more sensitive to 2,4-D than V2 and R2 growth stage, respectively. Previous studies reported greater reduction in the number of seeds plant⁻¹ when increasing rates of several synthetic auxin herbicides were sprayed at reproductive compared with vegetative growth stage (Solomon and Bradley 2014; Osipitan et al. 2019).

100 seed weight

Similarly, there was a reduction in 100 seed weight with increase in 2,4-D micro-rate across all DT soybean growth stages, Figure 2B. Interestingly, however, 100 seed weight response to increasing 2,4-D micro-rate was variable as was reported elsewhere (Solomon and Bradley 2014). For example, when 2,4-D micro-rate was increased from 0 to 1/1,000 of the label recommended rate (1.07 g ae ha⁻¹), reduction in 100 seed weight at R1 (7% from 35.8 to 33.3) was greatest and comparable to that at V2 (7% from 33.9 to 31.6) growth stage. Conversely, when 1/5 of the label recommended rate of 2,4-D (240 g ae ha⁻¹) was applied, R1 growth stage clearly had the greatest reduction in 100 seed weight (16%). On the other hand, both V2 and R2 experienced similar reduction in 100 seed weight (11%). Leon et al. (2014) attributed variability in peanut response to increasing rates of 2,4-D and dicamba to the complexity of their mode of action. Based on ED value estimates, a 2,4-D dose of 125.0, 85.4 and 27.5 g ae ha⁻¹ was required to reduce 100 seed weight by 5% (1.7 g) at V2, R1 and R2 growth stage, respectively, Table 2. This suggests, therefore, that the reproductive growth stages are more sensitive to 2,4-D than the vegetative one.

Soybean Yield Loss

In general, soybean yield loss increased with increase in 2,4-D micro-rate; however, the response was variable across all plant growth stages especially when DT soybean was sprayed with the lowest micro-rates of the herbicide, Figure 2D. For example, when 1/1,000 of the label

recommended rate of 2,4-D ($1.07 \text{ g ae ha}^{-1}$) was applied at V2, R1 and R2 growth stage, DT soybean had a 3.8, 3.4 and 3.4% yield loss, respectively. Conversely, when a 2,4-D dose twice as higher [$1/500$ of the label recommended rate ($2.14 \text{ g ae ha}^{-1}$)] was applied at V2, R1 and R2 growth stage, DT soybean had a much lower yield loss of 1.6, 1.9 and 2.5%, respectively.

On the other hand, when 2,4-D was applied at micro-rates equal and greater than $1/100$ of the label recommended rate, DT soybean yield loss response was more consistent and increased with increasing micro-rate of the herbicide. For example, $1/10$ of the label recommended rate of 2,4-D (107 g ae ha^{-1}) at V2, R1 and R2 growth stage DT soybean, caused 10, 51.3 and 13.9% yield loss, respectively. However, when DT soybean was treated with double the above micro-rate of the herbicide [$1/5$ of the label recommended rate (214 g ae ha^{-1})], yield loss was greatest with 22, 78 and 41% estimated at V2, R1 and R2 growth stage, respectively. This increase in yield in loss with micro-rate of 2,4-D is likely due to the negative impact the herbicide had on plant height (data not shown), yield components and other variables such as number of branches plant⁻¹ as discussed previously. Reduction in plant growth (e.g. height, number of branches plant⁻¹, and leaf area) is one of the major contributing factors to reduced yield components and can ultimately cause yield loss in soybean (Robinson et al. 2013; Costa et al. 2020). Based on ED value estimates, a 2,4-D dose of 18.3, 8.7 and $11.7 \text{ g ae ha}^{-1}$ was required to cause DT soybean a 5% (0.22 Mg ha^{-1}) yield loss at V2, R1 and R2 growth stage, respectively, Table 2. This suggests, therefore, that R1 is 2.1- and 1.3-times more sensitive to 2,4-D than V2 and R2 growth stage, respectively.

These findings are in agreement with what was discussed previously (Solomon and Bradley 2014; Osipitan et al. 2019) and further confirms that R1 is, indeed, the growth stage at which DT soybean is most sensitive to 2,4-D.

CONCLUSIONS AND IMPLICATIONS

Overall, results from this study showed that DT soybean response to 2,4-D micro-rates varied depending on plant growth stage at time of herbicide application. In general, soybean injury symptoms were only evident when 2,4-D was applied at $1/50$ (V2 and R2) and $1/100$ (R1) of the label recommended rate. Increase in 2,4-D micro-rate reduced soybean plant height and branching thus delaying canopy closure and reducing yield components (number of pods plant⁻¹ and 100 seed weight) which ultimately resulted in significant yield losses. Furthermore, results from this study showed that DT soybean is more sensitive to 2,4-D injury at reproductive than vegetative growth stages with R1 being the most sensitive one. It is worth mentioning, however, that DT soybean recovered from 2,4-D applications at vegetative growth stage much faster than reproductive ones. Therefore, care is needed to avoid any potential drift or tank contamination with 2,4-D especially at DT soybean reproductive growth stages.

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Table 1. Dose of 2,4-D that caused 5 (ED₅), 10 (ED₁₀) and 20% (ED₂₀) injury, delay in canopy closure, and reduction in the number of branches plant⁻¹ in dicamba-tolerant soybean at three growth stages. SE is standard error of the mean.

Parameter	Growth Stage	ED ₅ (SE)	ED ₁₀ (SE)	ED ₂₀ (SE)
Visual injury	V2	25.99 (1.08)	37.77 (1.57)	56.66 (2.35)
	R1	24.01 (1.29)	34.89 (1.88)	52.34 (2.82)
	R2	27.76 (1.17)	40.34 (1.70)	60.51 (2.55)
Days to canopy closure	V2	41.49 (12.18)	53.22 (15.62)	69.74 (20.48)
	R1	13.14 (4.45)	16.17 (5.48)	20.25 (6.86)
	R2	10.94 (6.39)	16.86 (8.23)	26.93 (10.46)
Branches plant ⁻¹	V2	63.44 (25.99)	80.13 (32.83)	103.24 (42.30)
	R1	13.99 (4.00)	16.58 (4.74)	19.94 (5.70)
	R2	146.50 (56.75)	180.30 (69.84)	225.85 (87.48)

Table 2. Dose of 2,4-D that caused 5 (ED₅), 10 (ED₁₀) and 20% (ED₂₀) reduction in dicamba-tolerant soybean pods plant⁻¹ and 100 seed weight, and yield loss at three growth stages. SE is standard error of the mean.

Parameter	Growth Stage	ED ₅ (SE)	ED ₁₀ (SE)	ED ₂₀ (SE)
Pods plant ⁻¹	V2	94.79 (29.05)	115.38 (35.36)	142.83 (43.78)
	R1	39.22 (20.09)	56.72 (29.06)	84.66 (43.38)
	R2	46.47 (27.62)	64.78 (38.50)	92.89 (55.20)
100 Seed weight	V2	125.04 (54.98)	164.91 (72.51)	222.68 (97.92)
	R1	85.39 (16.86)	112.62 (22.24)	152.07 (30.03)
	R2	27.49 (7.56)	39.24 (10.79)	57.74 (15.88)
Yield loss	V2	18.34 (6.45)	29.26 (10.29)	48.57 (17.08)
	R1	8.66 (2.26)	14.40 (3.77)	25.00 (6.54)
	R2	11.65 (2.48)	20.18 (4.30)	36.64 (7.80)

FIGURE LEGENDS

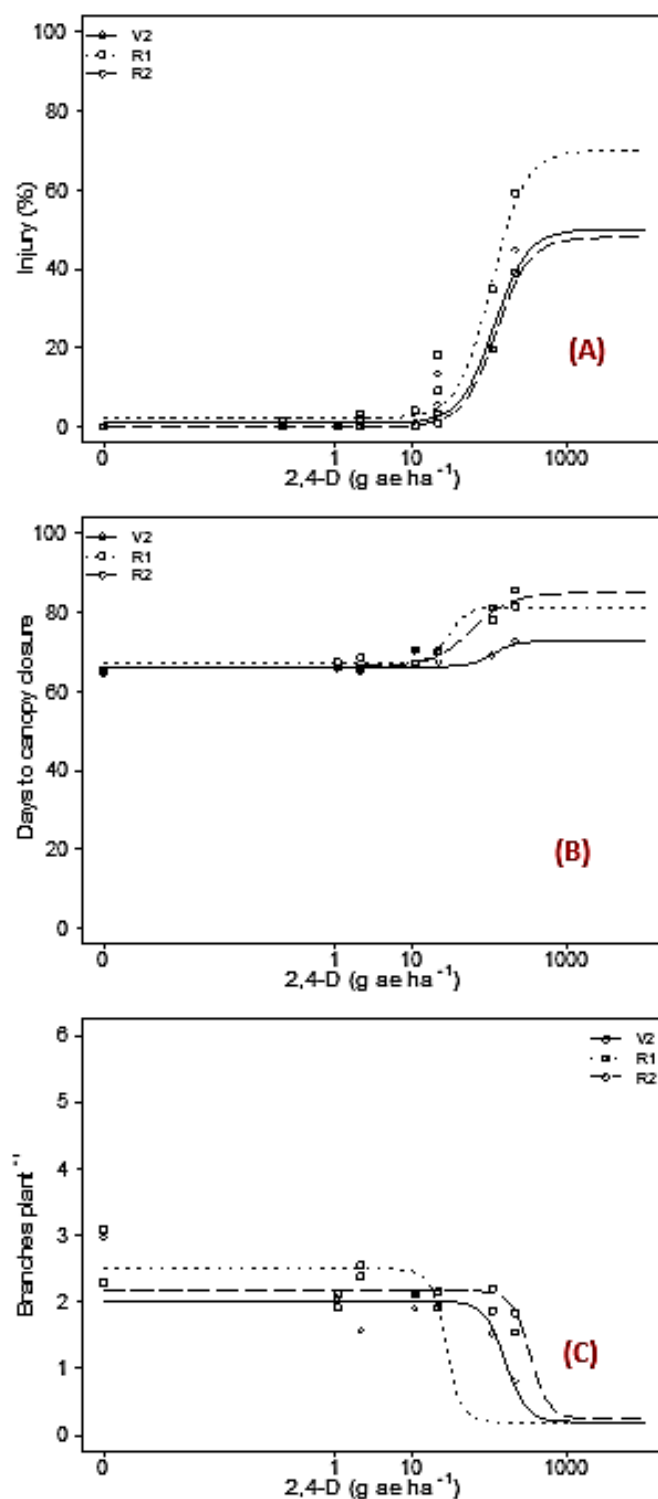


Figure 1. Dicamba-tolerant soybean (A) injury, (B) days to canopy closure, and (C) branches plant⁻¹ response to 2,4-D at three growth stages.

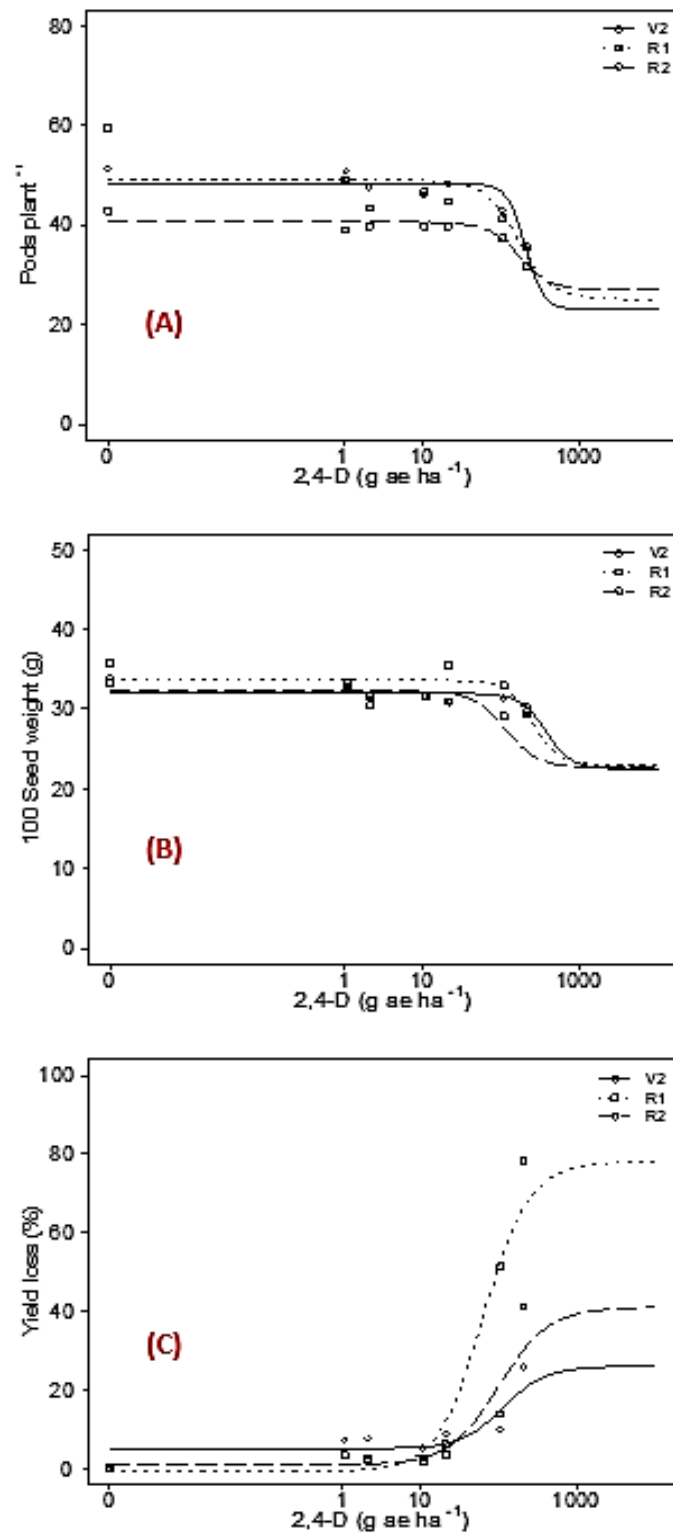


Figure 2. Dicamba-tolerant soybean (A) pods plant⁻¹, (B) 100 seed weight, and (C) yield loss response to 2,4-D at three growth stages.