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# Growth and Yield Losses of Roundup Ready Soybean as Influenced by Micro-rates of 2,4-D

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## Abstract

Widespread resistance to glyphosate has made weed control very challenging. In response, new approaches to managing resistant biotypes such as the Enlist E3™ have been developed. This technology allows in-crop use of 2,4-D but there is fear associated with unintentional application of the herbicide (e.g. direct application, tank contamination, or spray drift) to sensitive crops. A study was conducted to evaluate Roundup Ready (RR) soybean growth and yield losses as influenced by 2,4-D [six micro rates of 1/5, 1/10, 1/50, 1/100, 1/500 and 1/1000 of the 1,120 g ae ha<sup>-1</sup> label recommended dose, and a check with no herbicide applied] applied at V2, R1 and R2 growth stages. In general, RR soybean was more sensitive to 2,4-D at R1 than V2 and R2. The highest 2,4-D rate, 1/5 of the label recommended rate, caused 51% soybean injury symptom, 13 d canopy closure delay, 41.2% plant height reduction, and 68.9% yield loss at R1. Based on effective dose (ED) estimates, 37.7 g ae ha<sup>-1</sup> 2,4-D caused 5% yield loss (0.23 Mg ha<sup>-1</sup>) at R1 compared with a 2.5- and 2.0-fold higher dose at V2 and R2, respectively. With respect to number of days to canopy closure, both reproductive stages (R1 and R2) were equally less sensitive to 2,4-D than the vegetative one (V2) as the plants had already achieved maximum growth recorded. On the other hand, ED estimates for plant height have shown that both V2 and R2 were equally more sensitive to 2,4-D than R1. Altogether, these results clearly indicated that RR soybean growth and yield losses were significantly influenced by 2,4-D.

**Keywords:** Roundup Ready soybean, sensitivity, yield loss, 2,4-D

## 1. Introduction

The discovery of auxin type herbicides has revolutionized agriculture by shaping the way we farm in much of the world. Since their introduction in the mid-1940s, auxin herbicides not only have become one of the most widely used group of herbicides but also an integral component of major North America cropping systems. Auxin herbicides are very popular among growers, in part, because of their ability to selectively control broadleaf weeds including many herbicide-resistant ones (Heap, 2021). Although the evolution of weed resistance to auxin type herbicides may have been slow, the number of resistant species continues to increase. Just recently, Palmer amaranth, the most troublesome weed in the United States (U.S.), was reported to have evolved resistance to 2,4-D in two separate cases (Heap, 2019; Kumar et al., 2019). Although the reports indicate low resistance levels to 2,4-D, ranging from 3.2- to 10-fold, same biotypes had a high survival rate (81%) to dicamba in one case and multiple resistance to other herbicide modes of actions including acetolactate synthase-, protoporphyrinogen oxidase-, photosystem II- and 5-enolpyruvylshikimate-3-phosphate synthase-inhibitors in the other (Kumar et al., 2019). Regardless, synthetic auxins are still effective in controlling this weed. For example, in a recent study, Cuvaca et al. (2020) have demonstrated that dicamba is still effective in controlling Palmer amaranth provided that the herbicide is applied in a timely manner. Therefore, if further selection for synthetic auxins resistant biotypes is to be slowed, a more responsible use of these herbicides must be considered. This is especially relevant with the recent introduction of synthetic auxin tolerant crops in the U.S. One such example is the Enlist E3 soybean. This technology provides an additional tool for controlling herbicide-resistant weeds (Spaunhorst et al., 2014) and is genetically modified to resist 2,4-D herbicide, thus enabling in-crop use of Enlist herbicides, Enlist One and Enlist Duo. These compounds contain 2,4-D choline, which has the potential to

volatilize and move off-target. This is of major concern as an increase in the amount and frequency of use of this herbicide can potentially lead to unintentional movement and damage to sensitive crops (Egan et al., 2014). Soybean is very sensitive to 2,4-D even at sub-lethal doses (Egan et al., 201). Typical 2,4-D injury symptoms include twisting of stems (epinasty), strapping of leaves, and, in severe cases, plant death (Wax et al., 1969). Therefore, the risk of yield loss due to 2,4-D exposure is high. Previous studies have shown, for example, reduction in soybean seed weight and pod malformation following exposure to 2,4-D (Robinson et al., 2013; Solomon and Bradley, 2014). The objective of this study was, therefore, to evaluate Roundup Ready soybean growth and yield losses as influenced by micro-rates of 2,4-D at three growth stages.

## 2. Method

### 2.1 Experimental Site

Experiments were conducted at University of Nebraska Haskell Agricultural Laboratory near Concord, NE (HAL; 42.388, -96.957, 450 m) in 2019 and Eastern Nebraska Research and Extension Center near Mead, NE (ENREC; 41.151, -96.447; 355 m) in 2020. Conditions at both sites were described previously (Cuvaca et al., 2021).

### 2.2 Experimental Procedure

Experiments were conducted under irrigation utilizing a randomized complete block design with a split plot arrangement with 4 replications. Main plots evaluated seven 2,4-D (Enlist One) micro rates [0; 1/5; 1/10; 1/50; 1/100; 1/500; and 1/1000 of the 1,120 g ae ha<sup>-1</sup> label rate] and subplots evaluated 3 application times [V2 (2<sup>nd</sup> trifoliate), R1 (beginning bloom) and R2 (full bloom)]. Subplots were 7.6 m long with four rows of Roundup-Ready soybean each. The study was kept weed-free by hand-weeding as required throughout the growing season. Enlist One was applied using a CO<sub>2</sub>-pressurized backpack boom sprayer when wind speed was 6-9 km ha<sup>-1</sup> and air temperature was 19-22°C in 2019 and 21-25 °C in 2020.

### 2.3 Data Collection and Analysis

Roundup Ready soybean injury symptoms were visually assessed using a 0 (no injury) to 100 % (complete injury or plant death) scale at 7, 14 and 21 days after treatment (DAT). Other measurements collected were plant height (21 d after treatment), number of days to canopy closure, and number of branches plant<sup>-1</sup>. Soybean was combine harvested at physiological maturity, and yield loss was estimated. When year by treatment interaction was not detected, data were subjected to dose response analysis using the drc package in R version 3.4.1 (R Core Team, 2017). Roundup Ready soybean injury symptoms ratings, plant height, numbers of days to canopy closure, number of branches plant<sup>-1</sup> and yield loss were regressed over 2,4-D micro-rates using a four-parameter log-logistic equation (Knezevic et al., 2007). The regression analysis helped estimate the effective doses of 2,4-D (ED values) causing 5, 10 and 20% injury symptoms, growth reduction and yield loss at different growth stages and their respective standard errors.

## 3. Results and Discussion

Year effect was not significant; therefore, data were combined.

### 3.1 Soybean Injury Symptoms

Injury symptoms included epinasty, leaf bubbling and strapping, and reduction in plant height. The latter will be further discussed in the section that follows. In general, soybean injury symptoms increased with increase in 2,4-D micro-rate regardless of soybean growth stage at time of herbicide application. The degree of injury did, however, vary with soybean growth stage at time of herbicide application (Figure 1) as reported elsewhere (Solomon and Bradley, 2014; Osipitan et al., 2019). For example, increasing 2,4-D dose from 1/1000 to 1/5 of the label recommended dose resulted in soybean injury ranging from 0 to 48% at V2, 0 to 51% at R1, and 0 to 36% at R2 at 21 DAT, respectively. Based on ED value estimates, the V2 and R1 growth stages required equal but less amount of 2,4-D to cause the same injury level (e.g. 5, 10 and 20%) to RR soybean at 21 DAT compared with R2, Table 1. R1 was reported the most sensitive growth stage to dicamba exposure by Osipitan et al. (2019) who evaluated the response of Roundup Ready soybean to micro-rates of Engenia, XtendiMax and Clarity herbicides.

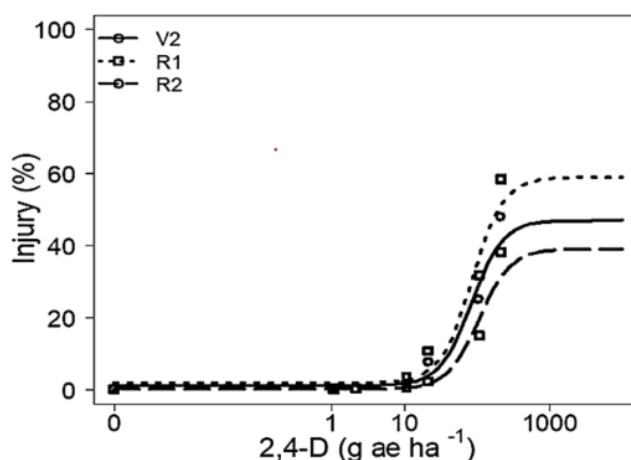


Figure 1. Roundup Ready soybean injury as influenced by 2,4-D at three growth stages

Table 1. Dose of 2,4-D required to cause 5 (ED<sub>5</sub>), 10 (ED<sub>10</sub>) and 20% (ED<sub>20</sub>) injury symptoms; delay in canopy closure; reduction in plant height, branch plant<sup>-1</sup>, flower plant<sup>-1</sup>, pods plant<sup>-1</sup>; and yield loss at three growth stages of Roundup Ready soybean

Parameter	Growth Stage	ED <sub>5</sub> (SE)	ED <sub>10</sub> (SE)	ED <sub>20</sub> (SE)
Injury symptoms	V2	19.3 (1.0)	28.1 (1.5)	42.1 (2.3)
	R1	19.6 (1.4)	28.4 (1.9)	42.6 (2.9)
	R2	24.5 (1.2)	35.6 (1.7)	53.3 (2.6)
Plant height (cm)	V2	19.2 (3.6)	26.5 (4.9)	37.6 (7.1)
	R1	38.2 (5.5)	46.6 (6.7)	57.9 (8.4)
	R2	18.0 (9.9)	21.4 (11.8)	25.8 (14.2)
Days to canopy closure	V2	13.9 (3.3)	16.6 (4.0)	20.3 (4.9)
	R1	49.2 (6.2)	59.6 (7.5)	73.4 (9.2)
	R2	44.9 (6.2)	53.5 (7.3)	64.7 (8.9)
Yield loss	V2	95.1 (35.6)	101.1 (19.7)	108.2 (9.2)
	R1	37.7 (5.4)	47.3 (6.7)	60.4 (8.6)
	R2	75.5 (18.9)	85.4 (15.5)	97.6 (11.4)

Note. SE is standard error of the mean.

### 3.2 Plant Height

In general, increase in 2,4-D micro-rate reduced RR soybean plant height with plant height reduction varying depending on soybean growth stage at time of herbicide application, Figure 2. For example, when RR soybean was treated with the lowest 2,4-D micro-rate [1/1000 of the label recommended dose of 2,4-D (1.07 g ae ha<sup>-1</sup>)] at V2, R1 and R2 growth stage, plant height was reduced only 0.6, 1.1 and 1%, respectively 21 DAT. However, when treated with the highest micro-rate of the herbicide (1/5 of the label recommended rate of 2,4-D) at V2, R1, and R2 growth stage, RR soybean plant height was reduced 9.3, 58.8 and 65.4%, respectively 21 DAT. Based on ED value estimates, more 2,4-D was required to reduce RR soybean plant height at R1 compared to V2 and R2 growth stages, Table 1. For example, 38.2 g ae ha<sup>-1</sup> 2,4-D was required to reduce RR soybean plant height by 5% at R1 compared to 19.2 g ae ha<sup>-1</sup> at V2 and 18.0 g ae ha<sup>-1</sup> at R2 growth stage.

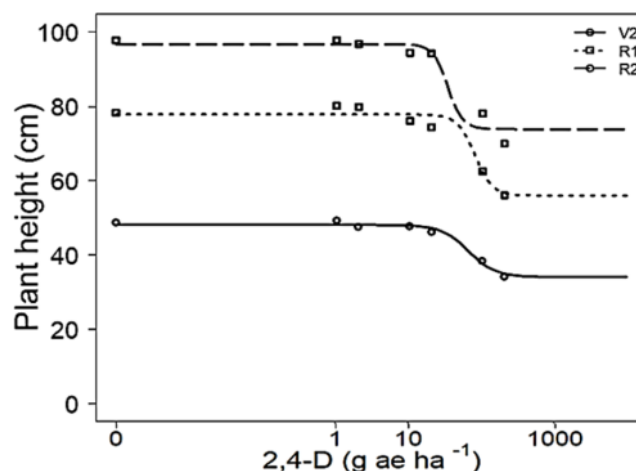


Figure 2. Roundup Ready soybean plant height as influenced by 2,4-D at three growth stages

### 3.3 Number of Days to Canopy Closure

On average, RR soybean closed canopy in 66.5 d. Increase in 2,4-D micro-rate delayed RR soybean canopy closure depending on growth stage at time of herbicide application. For example, increase in 2,4-D micro-rate from 0 to 1/1000 (lowest) of the label recommended rate delayed RR soybean canopy closure by 0.7 and 1.2 d at V2 and R1 growth stage, respectively but not at R2. When higher micro-rates of 2,4-D were applied, RR soybean canopy closure was further delayed regardless of growth stage at time of herbicide application, Figure 3. For example, when the highest micro-rate of 2,4-D (1/5 of the label recommended rate) was applied, RR soybean canopy closure was delayed by 12.9 d at V2, 13 d at R1, and 18.7 d at R2. This canopy closure delay with increase in 2,4-D micro-rate could be attributed to plant height reduction associated with stunted growth (data not shown). Previous studies have shown a delay in canopy closure as a result of stunted growth (Costa et al., 2020). Cuvaca et al. (2021) reported a delay in dicamba-tolerant soybean canopy closure with increase in 2,4-D micro-rate. Based on ED value estimates, RR soybean required 13.9 g ae ha<sup>-1</sup> of 2,4-D to cause 5% (3.3 d) delay in canopy closure at V2 but a 3.5- and 3.2-fold higher dose at R1 and R2, respectively (Table 1).

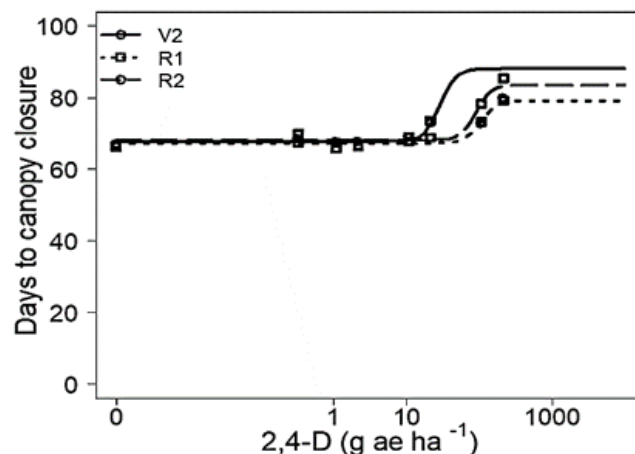


Figure 3. Roundup Ready soybean days to canopy closure as influenced by 2,4-D at three growth stages

### 3.4 Soybean Yield Loss

There was a significant reduction in RR soybean yield with increased 2,4-D micro-rates. Yield losses varied depending on plant growth stage at time of herbicide application, Figure 4. For example, 1/5 of the label recommended rate (214 g ae ha<sup>-1</sup>) applied at V2, R1 and R2 growth stage, caused 30.5, 68.9 and 46.9% yield loss, respectively. This reduction in RR soybean yield with increase in 2,4-D micro-rate was likely due to plant height reduction (Robinson et al., 2013). Based on the ED values estimates, the reproductive stages R1 and R2 were more sensitive to 2,4-D than the vegetative (V2) one, Table 1. For example, a 2,4-D dose of 95.1, 37.7 and 75.5 g

ae ha<sup>-1</sup> was required to cause 5% (0.23 Mg ha<sup>-1</sup>) yield loss at V2, R1 and R2 growth stage, respectively. This suggests that RR soybean is 2.5- and 2.0-fold more sensitive to 2,4-D at R1 than V2 and R2, respectively. Similar findings were reported previously (Cuvaca et al., 2021). This and other studies such as that of Osipitan et al. (2019) have shown that RR soybean is more sensitive to synthetic auxin herbicides at R1. Therefore, extra precaution should be taken when using these herbicides.

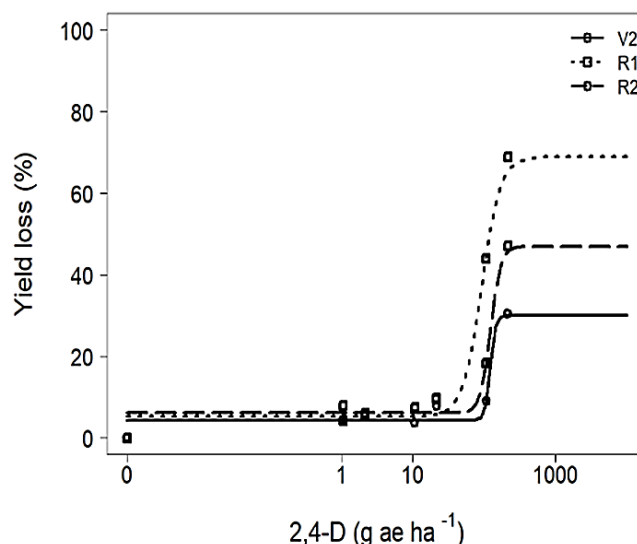


Figure 4. Roundup Ready soybean yield loss as influenced by 2,4-D at three growth stages

#### 4. Conclusion

Results showed that RR soybean growth and yield losses varied depending on 2,4-D micro-rate and plant growth stage at time of herbicide application. In general, RR soybean was more sensitive to 2,4-D at R1 than V2 and R2 growth stages. For example, a 2,4-D dose of 37.7 g ae ha<sup>-1</sup> was required to cause 5% yield loss (0.23 Mg ha<sup>-1</sup>) at R1 compared with a 2.5- and 2.0-fold higher dose at V2 and R2 growth stage, respectively. Therefore, extra precaution should be taken when using Enlist One herbicide especially around R1.

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