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Nebraska Soybean Board
FINAL Research Report Form

Note: Submit this report no later than 90 days after the NSB-funded project officially terminates.
This post-project 90-day time-frame will allow the Lead PI time to complete any final data analysis and a final technical report, plus the drafting of any articles for submission to scientific journals. Note that this completed report will be provided to the National Soybean Checkoff Research Database, (soybeanresearchdata.com).

Project # and Title: 1735: Impact of 2,4-D drift on non 2,4- D tolerant soybeans

Principal Investigator: Stevan Knezevic

Co-PI's & Institutions:

Project Date (Including Extension): 01/01/2019 ***to*** 12/30/2020 ***(For example: mm/dd/yyyy to mm/dd/yyyy)***

Total Budget for Project: $ 93,000.00

1. Briefly State the Rational for the Research:

With the increase in use of Enlist E3 soybean in the U.S., in-season application of 2,4-D (Enlist One and Enlist Duo) herbicides is of concern due to their potential off-target movement and potential threat to non 2,4-D tolerant soybean as well as other broadleaf crops. Furthermore, the negative impact of 2,4-D drift on non-2,4-D tolerant crops may vary with the rate of the herbicide, crop type and/or growth stage at time of drift occurrence. It is important to understand the potential impact of 2,4-D drift on non-2,4-D tolerant soybean. Therefore, a field study was conducted in 2019 and 2020 to measure the impact of 2,4-D micro-rates on four non 2,4-D tolerant soybeans (Dicamba-Tolerant, Roundup Ready, Liberty-Link and Conventional soybean).

2. Research Objectives: (copy from project, but keep in a brief bullet format)

1. To establish baseline data on the injury of four soybean types (Dicamba-Tolerant (DT), Roundup Ready, Liberty-Link, and Conventional) to six micro-rates of 2,4-D based herbicide (Enlist One).

3. General Approach Used and (if applicable) the Nebraska Test Locations:

Field studies were conducted in 2019 at Haskell Ag Lab, Concord, and in 2020 at Agronomy Farm by Mead, NE. The studies were laid out in a randomized complete block design with eight replications and a split-split-plot arrangement. The main plot treatments consisted of three application times [second trifoliate (V2); beginning of flowering (R1); and full flowering (R2)]; the sub plot treatments consisted of six micro rates of 2,4-D (1/5; 1/10; 1/50; 1/100; 1/500; and 1/1000 of the label recommended rate (32 oz/A) and a check with no herbicide applied; and the sub-sub plot treatments consisted of four soybean types (Dicamba-Tolerant, Roundup Ready, Liberty-Link, and Conventional). The first application time (V2) simulates early-season 2,4-D drift, and the remaining application times (R1 and R2) simulate late-season 2,4-D drift when planting in neighboring fields is delayed. Several factors can lead to delay in planting (e.g. logistics, weather). To assess the impact of potential 2,4-D drift, visual injury ratings (VIR) on a scale of 0 (no injury) to 100% (complete injury) and plant height (PLHT; inches) measurements were collected at 7, 14 and 21 days after treatment (DAT). In addition, soybean yields were also collected. All data were analyzed in R-statistical software by regressing VIR, PLHT and soybean yield over 2,4-D micro rates.

4. Describe Deliverables & Significance Attained for Each Research Objective:

Results from this project were used to make a series of deliverables, which included: posters and oral presentations for professional Weeds Science Society conferences, extension articles published in Crop Protection Clinics, Wed-Based Crop Watch, Trade Journals and Local Newspapers as well as several scientific manuscripts. Also, whole series of Power-Point presentations were developed and delivered during many extension programs across Nebraska over last 2 years.

The list of significant learnings from the project included:

1. Dicamba-Tolerant (DT), Roundup Ready, Liberty-Link, and Conventional soybeans were equally sensitive to all tested micro-rates of 2,4-D. Due to limited space in this report, the results from DT-soybeans are presented only. For more details, please see Technical Report.

2. Many crop growth parameters were significantly impacted, including reductions in plant height by as much as 24” (due to crop stunting) and delay in days to canopy closure from 2 to as much as 20 days.

3. Visual injuries were evident from all rates of 2,4-D, ranging from 5-60% across all three application times. Highest injuries were observed at beginning of flowering (R1) versus second trifoliate (V2) and full flowering (R2) stages (V2 and R2 stages have resulted in similar injury levels). Injuries were visible in the form of: leaf bubbling, strapping and bending of petioles and branches.

4. Describe Deliverables & Significance Attained for Each Research Objective (continued)

4. Yield of all four soybean types were significantly affected by all three application time. Yields of DT-soybeans ranged from 67-69 bu/A in non-sprayed control plots to as low as 38 bushel in heavy injured plots.

5. Yield losses varied from 5% - 80% depending on the 2,4-D dose and application time. The R1 stage was the most sensitive stage, followed by R2 and then V2 stage.

When DT-soybean was treated with 1/10 of the label dose of 2,4-D (3.2 oz A-1) at V2, R1 and R2 stage, yield loss were 10, 51 and 14%, respectively. Furthermore, when 1/5 of the label dose (6.4 oz A-1) was applied at V2, R1 and R2 stage, the resulting yield loss was further increased to 26, 78 and 42%, respectively, thus confirming that R1 is the most sensitive stage.

The 2,4-D threshold dose of 0.55, 0.25 and 0.34 oz per acre caused 5% yield loss (threshold) treated at V2, R1 and R2 stage, respectively. Therefore, it is important to prevent potential 2,4-D drift onto non-2,4-D tolerant soybeans.

5. List where the Project Research Results/Findings were Publicized:

Presented 4 posters in 2019 and 2 posters at 2020 North Central Weeds meetings (Virtual conference).
- Presented 1 talk at International Plant Science Conference in Rome.
- Presented 2 posters and 1 talk in 2020 at the WSSA meetings
- Presented 2 posters in 2019 at the Western Society of Weeds.
- Total of about 22 Power Point presentations for use during UNL-Extension Meetings, and Farm Shows, which are attended by farmers, crop consultants, and Ag folks in general.
- Multiple articles in UNL Web-based Crop-Watch Newsletter and local papers (Norfolk, Wayne)
- Currently Developing 4 scientific manuscripts:

1. Cuvaca I and Knezevic, S.Z. (2021). "Glyphosate-Tolerant Soybean Response to Microrates of 2,4-D.
2. Cuvaca I and Knezevic, S.Z. (2021). "Liberty-Link Soybean Response to Microrates of 2,4-D".
3. Cuvaca I and Knezevic, S.Z. (2021). "Dicamba-Tolerant Soybean Response to Microrates of 2,4-D".
4. Cuvaca I and Knezevic, S.Z. (2021). "Conventional Soybean Response to Microrates of 2,4-D".

Note: The above boxes will automatically accomodate for your text inputs; HOWEVER, the Final Report comprised of the above listed items must be kept to THREE PAGES. A Technical Report of no more than TEN PAGES (preferably fewer) can be appended to this report.
Submit both reports as a single PDF with this file name format: #XXX > FINAL > Project Title > PI last name

Please email this completed form to the Agriculture Research Division (jmcmahon10@unl.edu) based on the reporting schedule given to you. If you have any questions, please call Jen McMahon at the ARD at 2-7082.

Final Technical Report

Project Title:

Impact of 2,4-D drift on Non 2,4-D-Tolerant Soybeans

Investigator:

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Introduction: With the increase of acres planted with Enlist E3 soybean in the U.S., in-season application of 2,4-D (Enlist One and Enlist Duo) herbicides is of concern due to their potential off-target movement and potential threat to non 2,4-D tolerant soybean as well as other broadleaf crops. Furthermore, the negative impact of 2,4-D drift on non-2,4-D tolerant crops may vary with the rate of the herbicide, crop type and/or growth stage at time of drift occurrence. It is important to understand the potential impact of 2,4-D drift on non-2,4-D tolerant soybean. Therefore, a field studies were conducted to measure the impact of 2,4-D micro-rates on four non 2,4-D tolerant soybeans (Dicamba-Tolerant, Roundup Ready, Liberty-Link and Conventional soybean).

Study Procedure: Four field studies were conducted near Concord, NE in 2019 and at Mead, NE in 2020 to investigate soybean response to 2,4-D micro-rates at three growth stages. Each experiment consisted of one soybean type, including Dicamba-Tolerant (DT), Roundup Ready (RR), Liberty-Link (LL) and Conventional (C) soybean. The experiments were laid in a randomized complete block design with six replications and a split-plot arrangement of treatments. Main plots consisted of three 2,4-D application times [second trifoliate (V2); beginning of flowering (V7/R1); and full flowering (R2)]. Subplots consisted of six micro rates of 2,4-D (1/5; 1/10; 1/50; 1/100; 1/500; and 1/1000 of the label recommended dose of 1,120 g ae ha-1) and a check with no herbicide applied. The first application time (V2) simulates early-season 2,4-D drift, and the remaining application times (R1 and R2) simulate late-season 2,4-D drift when planting in neighboring fields is delayed. To assess the impact of potential 2,4-D drift, visual injury ratings (VIR) on a scale of 0 (no injury) to 100% (complete injury), plant height (in), days to canopy closure and yield were collected. Data were analyzed using a 4 parameter log logistic (LL.4) equation in R-statistical software, and effective doses (ED) of the herbicide required to cause 5, 10 and 20% injury, reduction in plant height, delay in canopy closure and yield loss were estimated.

<u>Results:</u> The 2019 and 2020 data did not differ statistically, therefore they were combined for each soybean type. Dicamba-Tolerant (DT), Roundup Ready, Liberty-Link, and Conventional soybeans were equally sensitive to all tested micro-rates of 2,4-D. Due to limited space in this report, the results from DT-soybeans are presented only. Our plan for 2021 is to publish four manuscripts, one manuscript for each soybean type, which can be shared with the Board if inquired from me.

Visual Injury: In general, DT soybean treated with 2,4-D exhibited injuries that ranged from leaf bubbling, and strapping to bending of petioles and branches. Increase in 2,4-D micro rates increased DT soybean injuries regardless of application time. Highest injuries were observed when DT soybean was treated with 2,4-D at beginning of flowering (R1) versus second trifoliate (V2) and full flowering (R2) stages (Figure 1). 2,4-D applications at the V2 and R2 stages have resulted in similar injury levels (Figure 1). For example, DT soybean treated with 1/100 of the label recommended dose of 2,4-D (0.32 oz A^{-1}) at R1 had 4% injury but no injury at all when treated at V2 and R2 stages. However, when DT soybean was treated with 1/5 of the label recommended dose of 2,4-D (6.4 oz A^{-1}) at V2, R1 and R2 stage, visual injuries were 45, 59 and 39% respectively. Based on ED value estimates, a 2,4-D dose of 0.71 oz A^{-1} was required to cause 5% injury to DT soybean at R1 at 21 d after treatment (DAT) but a 1.1- and 1.2-fold higher dose of the herbicide was required to cause the same injury level at V2 and R2, respectively (Table 1). Similarly, a 1.1- and 1.2-fold higher dose of 2,4-D was required to cause 10% injury to DT soybean treated at V2 and R2 stage, respectively, compared with R1. These results suggest, therefore, that DT soybean is more sensitive to 2,4-D at R1 than V2 and R2 stages (Tables 1).

Plant Height: There was a reduction in plant height of DT soybeans with increase in 2,4-D micro-rates regardless of growth stage at time of herbicide application (Figure 2). For example, increase in 2,4-D micro-rate from 0 to 1/100 of the label recommended dose (0.32 oz A^{-1}) reduced DT soybean height from 20 to 17.57 in, 31.8 to 27.9 in, and 39.6 to 34.2 in at V2, R1 and R2 stage, respectively. When DT soybean was treated with 1/5 of the label recommended dose of 2,4-D (6.4 oz A^{-1}) at V2, R1 and R2 stage, plant height was further reduced to 14.1, 19.3 and 24.3 in, respectively. This reduction in plant height was due to a stunted growth (Figure not shown) following 2,4-D application. Based on ED value estimates, a 2,4-D dose of 0.54, 0.56 and 0.29 oz A^{-1} was required to reduce DT soybean height by 5% at V2, R1 and R2 stage, respectively. These estimates suggest that DT soybean is more sensitive to 2,4-D at later growth stages with R2 being 1.9-times more sensitive to the herbicide than V2 and R2 stages (Table 1).

Days to Canopy Closure: Untreated DT soybean reached canopy closure in 64.9 d, on average. DT soybean treated with 2,4-D, on the other hand, had a stunted growth which ultimately delayed canopy closure regardless of plant growth stage at time of herbicide application (Figure 3). For example, when DT soybean was treated with 1/1000 of the label recommended dose (0.032 oz A^{-1}) at V2, R1 and R2 stage, canopy closure was delayed by 1.1, 1.3 and 2.1 d, respectively. When DT soybean was treated with 1/5 of the label recommended dose of 2,4-D (6.4 oz A^{-1}) at V2, R1 and R2 stage, canopy closure was further delayed by 8.3, 16.8 and 20.2 d, respectively. This suggests that DT soybean is more sensitive to 2,4-D at reproductive than vegetative stages. Based on ED value estimates, a 2,4-D dose of 0.32, 0.39 and 1.24 oz A^{-1} was required to delay DT soybean canopy closure by 5% (3.2 d) at V2, R1 and R2 stage, respectively (Table 1) thus further suggesting 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 the V2 stage.

Soybean Yield: Yield of DT soybean was significantly impacted by 2,4-D micro-rates regardless of application time. Differences between application times became clearer with increase in 2,4-D micro-rate, with later growth stages being impacted the most (Figure 4). For example, when DT soybean was treated with the lowest dose of 0.032 oz A^{-1} 2,4-D (1/1000 of the label recommended dose) at V2, R1 and R2 stage, yields were 69.1, 67.0 and 69.7 bu A^{-1}, respectively. However, when DT soybean was treated with the highest dose of 6.4 oz A^{-1} 2,4-D (1/5 of the label recommended dose) at V2, R1 and R2 stage, yields were 57.9, 38.6 and 49.8 bu A^{-1}, respectively. Based on ED value estimates (Table 1), a 2,4-D dose of 0.60, 0.47 and 1.2 oz A^{-1} was required to reduce DT soybean yield by 5% at V2, R1 and R2 stage, respectively. This suggests that DT soybean is 1.3- and 2.5-fold more sensitive to 2,4-D at R1 than V2 and R2 stage, respectively.

Soybean Yield Loss: In general, there was an increase in DT soybean yield loss with increase in 2,4-D micro-rate regardless of plant growth stage at time of herbicide application (Figure 5). DT soybean treated with 1/1000 of the label recommended dose of 2,4-D (0.032 oz A^{-1}) had a yield loss of 3.4% across all growth stages. Overall yield losses were higher at reproductive than vegetative stages (Figure 5). When DT soybean was treated with 1/10 of the label recommended dose of 2,4-D (3.2 oz A^{-1}) at V2, R1 and R2 stage, yield loss were 10, 51.3 and 13.9%, respectively. When DT soybean was treated with 1/5 of the label recommended dose of 2,4-D (6.4 oz A^{-1}) at V2, R1 and R2 stage, the resulting yield loss was further increased to 25.7, 78.3 and 41.1%, respectively thus suggesting that R1 is the most sensitive stage. Based on ED value estimates (Table 1), a 2,4-D dose of 0.55, 0.25 and 0.34 oz A^{-1} was required to cause 5% yield loss in DT soybean treated at V2, R1 and R2 stage, respectively thus further suggesting that DT soybean is more sensitive to 2,4-D at reproductive than vegetative stages with R1 being 2.2- and 1.4-times more sensitive to the herbicide than V2 and R2 stage, respectively. Altogether, these results suggest that DT soybean is more sensitive to 2,4-D at R1 than other growth stages.

Summary, Conclusions and Implications: Results from this study clearly showed that DT soybean is sensitive to 2,4-D; however, the degree of sensitivity seems to be dependent on growth stage at time of exposure. DT soybean is more sensitive to 2,4-D at R1stage. Increase in DT soybean injury and reduction in plant height with increase in 2,4-D micro-rate clearly delayed canopy closure and reduced grain yield. This is a result, in part, from stunted growth following exposure to 2,4-D. Potential implications of this includes reduced crop competitiveness against weeds. It is also worth noting, however, that flowering and maturity were delayed (data not shown) which could potentially pushback harvesting as well as planting of subsequent crops in double crop soybean systems. Delaying harvesting, on the other hand, could subject soybean to early frost damage. Therefore, it is important to prevent potential 2,4-D drift onto non-2,4-D tolerant soybean.

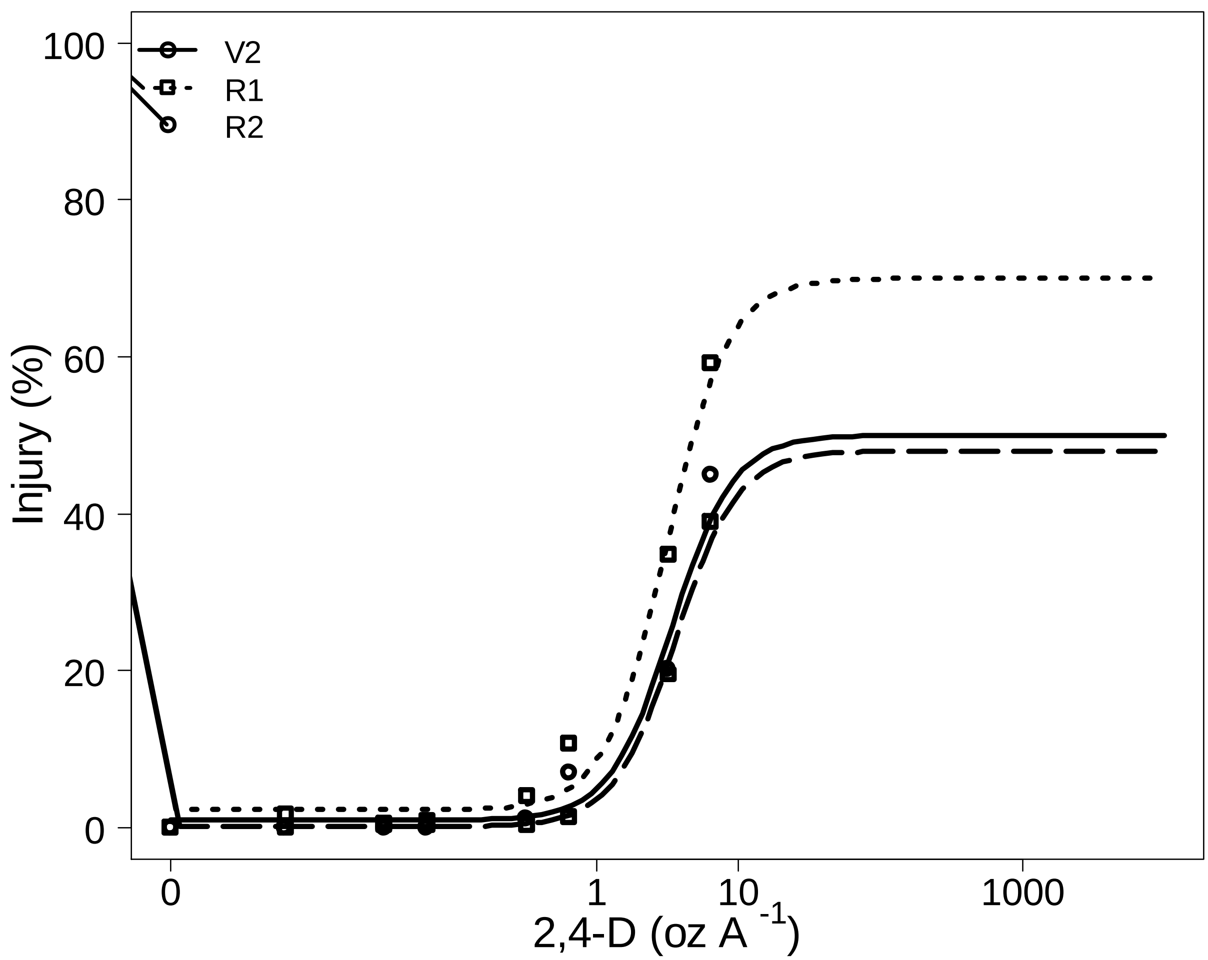

Figure 1. Dicamba-tolerant soybean injury at 21 days after treatment with 2,4-D micro-rates at second trifoliate (V2), beginning of flowering (R1) and full flowering (R2) stages

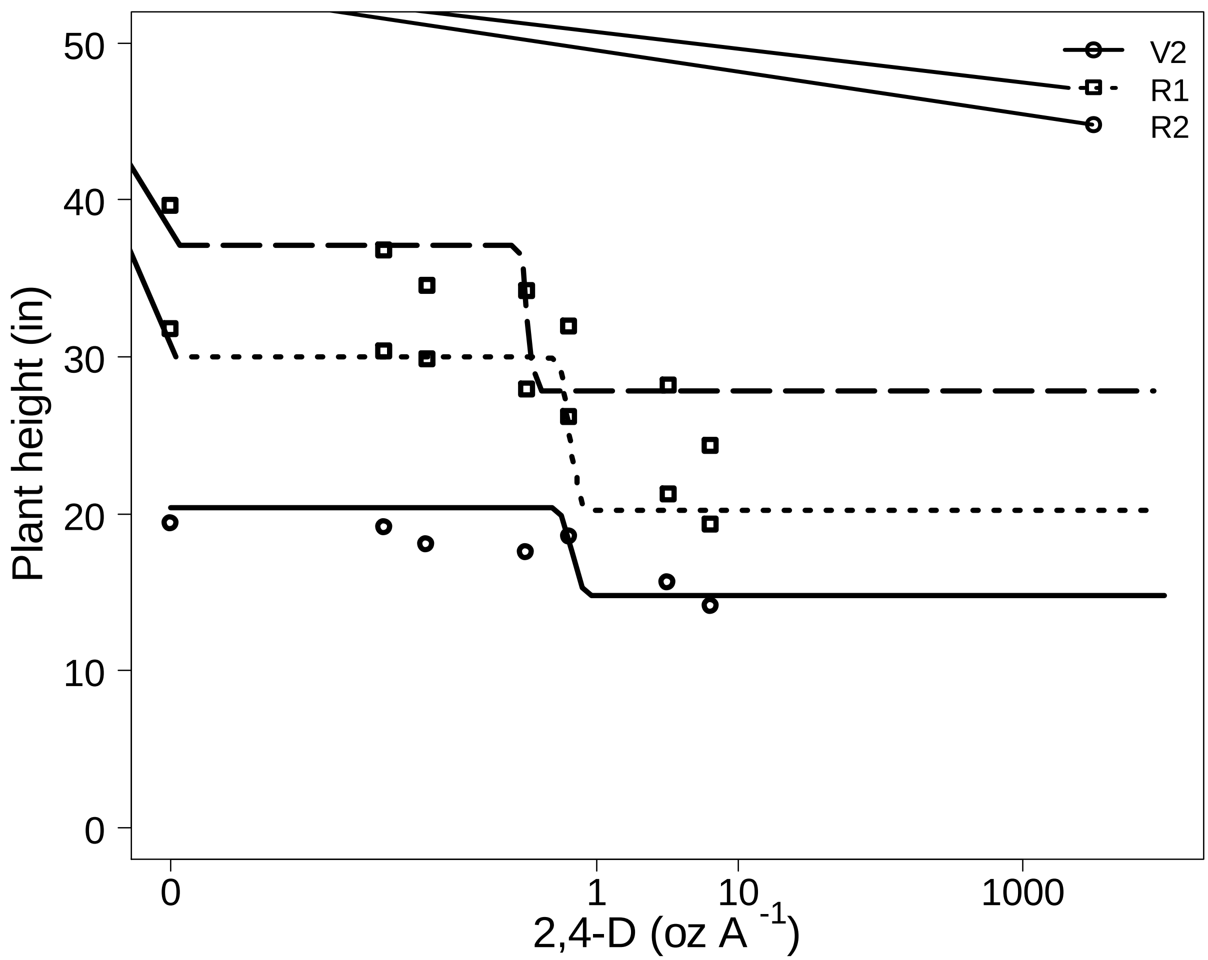

Figure 2. Dicamba-tolerant soybean height at 21 days after treatment with 2,4-D micro-rates at second trifoliate (V2), beginning of flowering (R1) and full flowering (R2) stages.

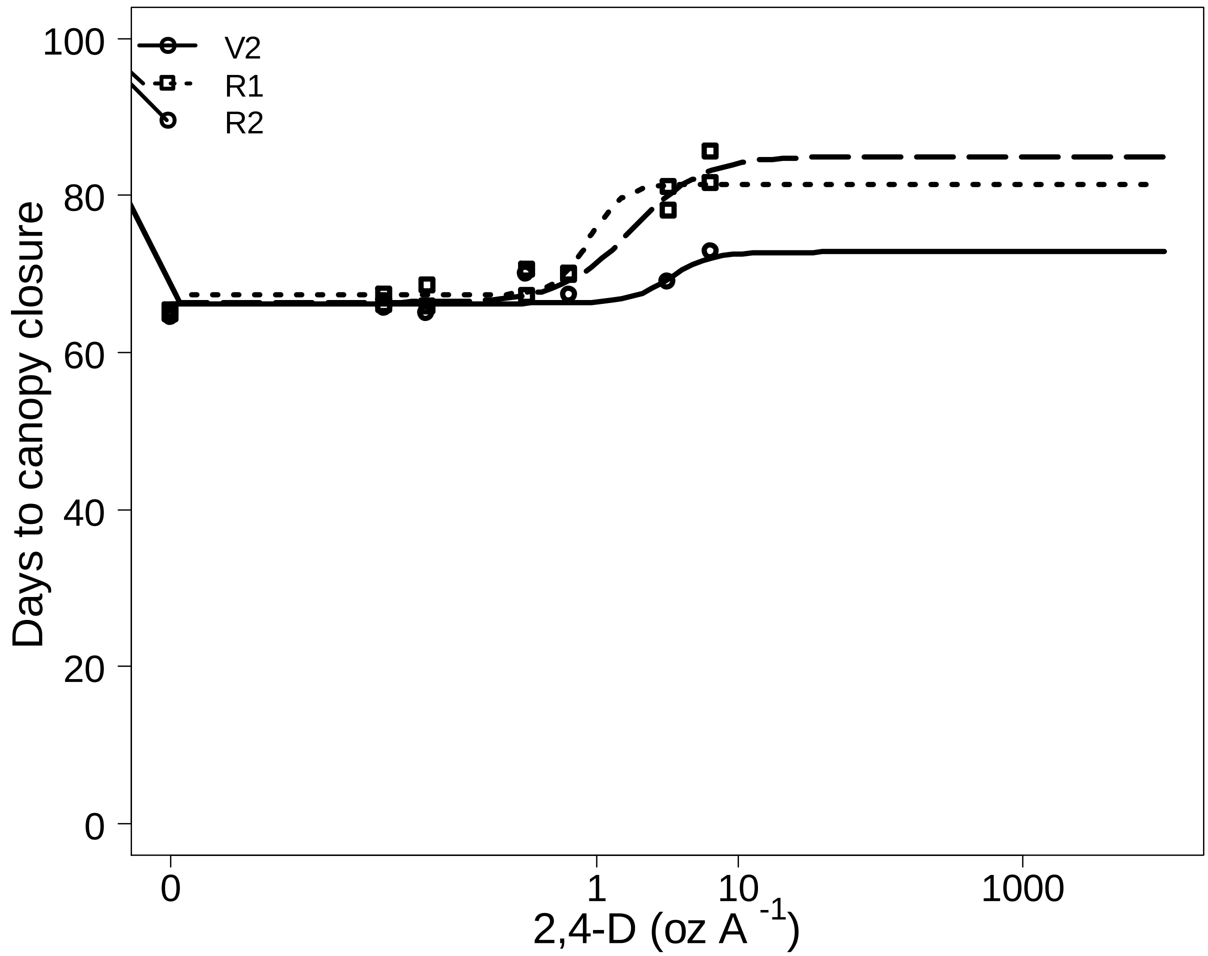

Figure 3. Dicamba-Tolerant soybean days to canopy closure as influenced 2,4-D micro-rates at second trifoliate (V2), beginning of flowering (R1) and full flowering (R2) stages.

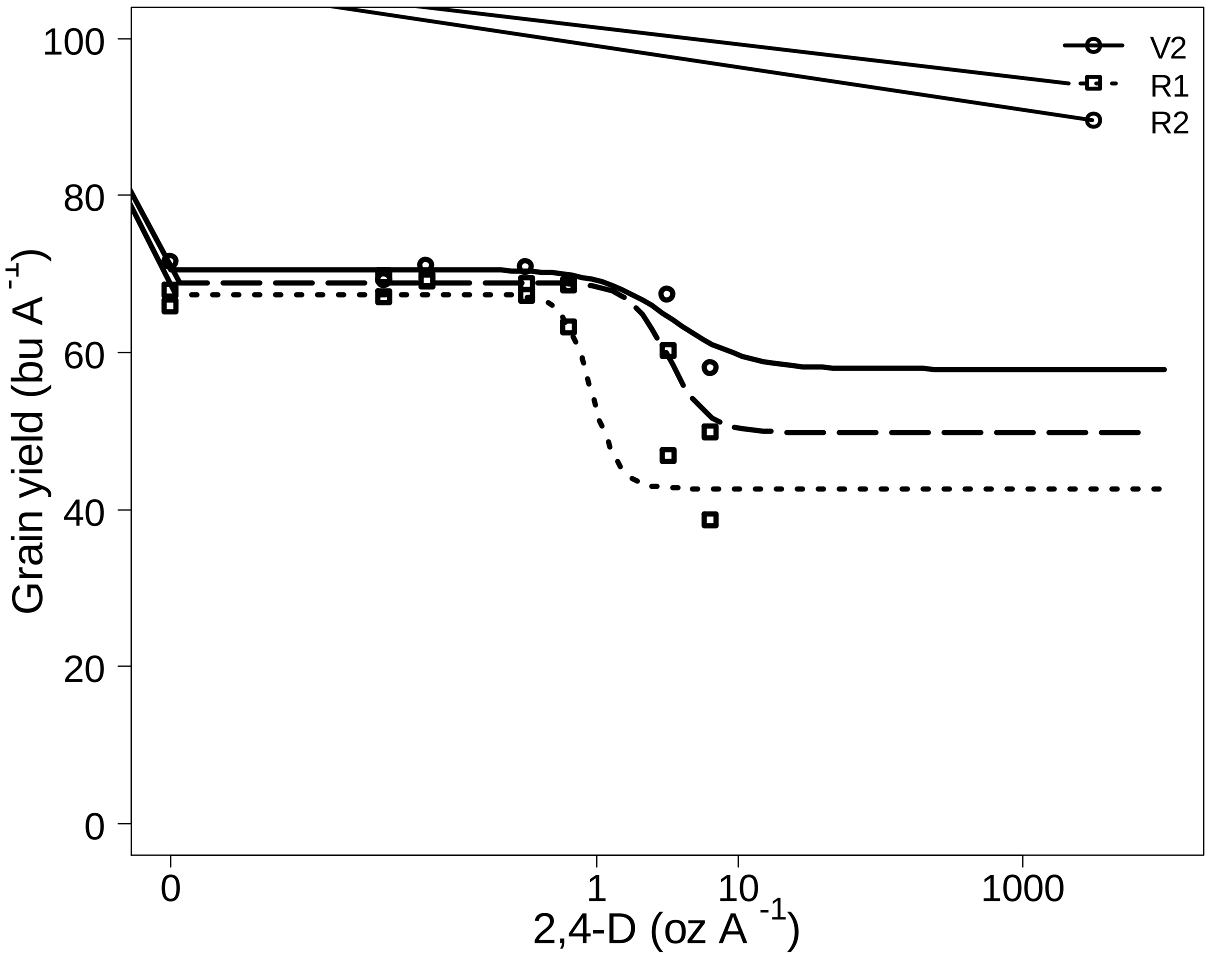

Figure 4. Dicamba-Tolerant soybean yield response to 2,4-D micro-rates at second trifoliate (V2), beginning of flowering (R1) and full flowering (R2) stages.

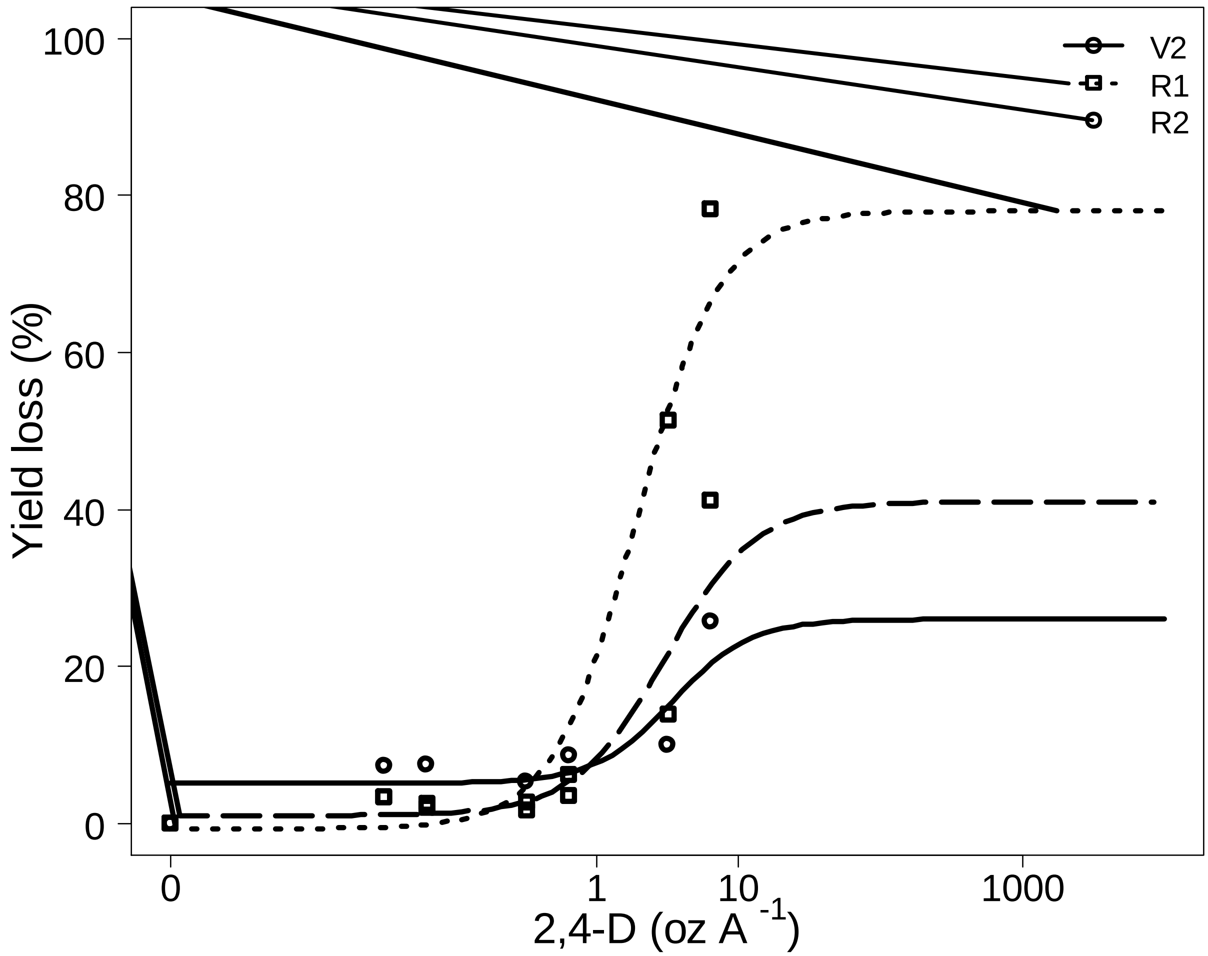

Figure 5. Dicamba-Tolerant soybean yield loss as influenced by 2,4-D micro-rates at second trifoliate (V2), beginning of flowering (R1) and full flowering (R2) stages.

Table 1. Dose of 2,4-D that resulted in 5, 10 and 20% injury and reduction in plant height at 21 d after treatment, delay in canopy closure, and yield reduction in Dicamba-Tolerant soybean at three growth stages

Variable	App/stage	ED_5 (SE)	ED_{10} (SE)	ED_{20} (SE)
		----------------------- 2,4-D in oz A^{-1}	-----------------------	
Visual injury	V2	0.78 (0.03)	1.13 (0.04)	1.69 (0.07)
	R1	0.71 (0.03)	1.04 (0.05)	1.56 (0.08)
	R2	0.83 (0.03)	1.20 (0.05)	1.80 (0.07)
Plant height	V2	0.54 (0.02)	0.57 (0.02)	0.61 (0.02)
	R1	0.56 (0.01)	0.58 (0.01)	0.60 (0.01)
	R2	0.29 (0.01)	0.30 (0.01)	0.31 (0.01)
Day(s) to canopy closure	V2	1.24 (0.36)	1.59 (0.46)	2.08 (0.61)
	R1	0.39 (0.13)	0.48 (0.16)	0.60 (0.20)
	R2	0.32 (0.19)	0.50 (0.24)	0.80 (0.31)
Yield	V2	0.60 (0.22)	0.93 (0.34)	1.51 (0.55)
	R1	0.47 (0.09)	0.56 (0.11)	0.67 (0.13)
	R2	1.24 (0.16)	1.58 (0.21)	2.06 (0.27)
Yield loss	V2	0.55 (0.19)	0.87 (0.30)	1.45 (0.51)
	R1	0.25 (0.06)	0.43 (0.11)	0.74 (0.19)
	R2	0.34 (0.07)	0.60 (0.12)	1.09 (0.23)