

Effect of Low Rate of Dicamba on Tomato (*Solanum lycopersicum*) at Different Growth Stages

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Received: July 15, 2023

Accepted: August 20, 2023

Online Published: September 15, 2023

doi:10.5539/jas.v15n10p9

URL: <https://doi.org/10.5539/jas.v15n10p9>

Abstract

Tomatoes are highly sensitive to herbicides, and concerns have been raised regarding off-target movement of dicamba and 2,4-D with the advent of new technologies in crops like soybean and cotton. Greenhouse studies were conducted over two years to assess the effect of low rates of dicamba on tomatoes at different growth stages and investigate fruit contamination. Treatments included untreated controls and dicamba applied at 1/16X, 1/32X, 1/64X, and 1/128X rates with non-ionic surfactant (NIS). Tomato plants at three growth stages (vegetative, flowering, and fruiting) were evaluated for dicamba sensitivity. Vegetative stage plants showed the highest sensitivity, while no significant differences in injury were observed between flowering and fruiting stages. Only the untreated controls produced fruit at the vegetative stage. Plants at flowering and fruiting stages successfully produced fruits. Harvested tomato fruits from each dicamba rate and the untreated control were planted, and progeny (F1) seedlings were evaluated for dicamba symptomology. No visual dicamba symptoms were observed in the tomato progeny, indicating the absence of dicamba contamination. High-performance liquid chromatography analysis confirmed no detectable levels of dicamba in the fruit samples. These findings indicate that low rates of dicamba, even at simulated drift levels, do not significantly affect tomatoes or result in fruit contamination. The results contribute to understanding the risks associated with herbicide drift and its impact on sensitive crops like tomatoes.

Keywords: tomato, vegetative, flowering, fruiting, seedling, progeny, dicamba, drift, HPLC

1. Introduction

Tomatoes (*Solanum lycopersicum*) are a vital agricultural crop in the United States, with California being the leading tomato-producing state. In 2019, the U.S. produced approximately 32 billion pounds of tomatoes, contributing significantly to the economy through revenue generation (Parr et al., 2019). While the majority of tomato production is for processing purposes, a smaller proportion is dedicated to fresh market sales. Florida, Ohio, and Indiana are also prominent tomato-producing states. In Mississippi, tomato cultivation plays a crucial role in the state's agricultural industry and economy. With its warm climate and fertile soils, Mississippi offers favorable conditions for tomato production (USDA, 2017). The state ranks among the top 10 tomato-producing states in the U.S., emphasizing the significance of this crop in the region.

Dicamba is a widely-used herbicide in soybean and cotton crops that has been found to cause significant damage to non-target crops, including tomatoes (USGS, 2021). The drift of dicamba from treated fields onto neighboring tomato plants can lead to yield losses, reduced fruit quality, and even complete crop failure (USEPA, 2021; Zangouejinejad et al., 2019). Dicamba drift occurs when the herbicide volatilizes, or becomes a gas, and moves off-target due to wind or other factors (Behrens & Lueschen, 1979). The use of Xtend technology, which allows for the application of dicamba on soybean and cotton crops, has been identified as a major contributor to dicamba drift onto tomato plants (Reed, 2020). This is because the herbicide can remain active in the air for up to several days after application, increasing the risk of drift onto neighboring crops. Xtend technology is a type of genetic modification that has been developed for soybean and cotton crops (USEPA, 2016). This technology allows these crops to be resistant to certain herbicides, which can help to increase yield and minimize damage from weeds. However, there are concerns about the potential drift of these herbicides onto other crops, such as tomato plants. Research has shown that the use of Xtend technology in soybean and cotton can lead to herbicide drift onto tomato

plants, which can cause significant damage to the fruit (Meyers et al., 2022). This highlights the importance of careful management practices and monitoring of herbicide application to minimize the risk of drift onto non-target crops. Dicamba drift can cause a range of negative effects on tomato plants, including stunted growth, leaf cupping, fruit malformation, and reduced yield (Kruger et al., 2012). ‘Money Maker’ and Better Boy tomato crops at 28 DAT suffered crop injury as high as 89 and 99%, respectively, when exposed to 3 g ha⁻¹ of dicamba (Zangouinejad et al., 2019). The severity of these effects depends on the timing and amount of dicamba exposure, as well as the age and variety of the tomato plants. In some cases, dicamba drift can result in the complete loss of the tomato crop, leading to significant economic losses for growers.

Dicamba herbicide usage raises concerns due to its potential translocation into tomato fruits, which can pose risks to consumer safety. Meyers et al. (2022) detected dicamba residue in tomato fruit when plants were treated with 53 g ha⁻¹ at 61 days after treatment (DAT). Kruger et al. (2012) reported that glyphosate and dicamba herbicides exhibited a dose-dependent effect on tomato plants, with higher concentrations resulting in increased injury. Glyphosate displayed a stronger impact compared to dicamba, and the injury severity was enhanced when both herbicides were applied simultaneously. Jones et al. (2017) found that dicamba herbicide exposure during early growth stages of soybean plants led to herbicide translocation within the plant, resulting in reduced progeny yields. The degree of yield reduction varied based on herbicide concentration and application timing, emphasizing the importance of prudent dicamba herbicide management to mitigate negative effects on soybean crops. Griffin et al. (2013) demonstrated that the detrimental effects of dicamba herbicides on soybean crops varied depending on the growth stage. Early applications during the V2 to V4 growth stages had the greatest impact on yield, while later applications during the R1 to R3 growth stages exhibited a diminished effect. In this study, our objectives were to determine the effect of a low-rate, simulated drift-rate application of dicamba on tomatoes at different growth stages, to investigate dicamba translocation to tomato fruits and assess its potential implications for fruit quality and safety (evaluate potential contamination of the fruit).

2. Materials and Methods

Two years of greenhouse studies were conducted at the Delta Research and Extension Center in Stoneville, Mississippi in 2021 and 2022. The greenhouse temperatures were 32, 25, 22, 25, and 27 °C and relative humidity was 55, 45, 45, 40, 44, and 45% in September, October, November, December, January, and February, respectively. The experiment was designed as three (growth stage) by five (treatments) factorial arrangement in a randomized complete block and replicated four times. Treatments were as follows: 1) untreated control; 2) dicamba at 1/16X rate (0.0731 L/ha) + Non-ionic surfactant (NIS) at 0.25% (v/v); 3) dicamba at 1/32X rate (0.0366 L/ha) + NIS; 4) dicamba (clarity) at 1/64X rate (0.0183 L/ha) + NIS; and 5) dicamba at 1/128X rate (0.0091 L/ha) + NIS (Griffin J. L. et al. 2013). The 1X rate of dicamba is 1.17 L/ha. The three-growth stage of tomato (application timing) were: A) vegetative stage (before flowering) (Principal growth stage 1: Leaf development; BBCH (Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie) code 14: fourth pair of true leaves visible); B) at flowering (Principal growth stage 6: Flowering; BBCH code 60: first flowers open); and C) at fruiting (Principal growth stage 7: fruit development; BBCH code 70: Fruits at the main stem or branches visible) (Cardoso et al. 2021). Tomato injury was scored on a scale of 0 to 100 (0 being no injury and 100 being complete crop death). The visual rating for crop injury was made based on the whole tomato plant treated with dicamba in comparison to the non-treated control pot.

2.1 Year One

In the year one study, tomato (cherry tomato) seeds were planted in small pots (6.4 × 6.4 × 7.6 cm) containing putting-mix (commercial putting-mix: Berger BM6 All Purpose Peat Mix, 3 cu. Ft. KBWsupply.com) on January 20, 2021, and tomatoes emerged on January 28. Tomato seedlings were transplanted in a bigger pot (10.2 × 10.2 × 12 cm) on February 17. Tomato plants were watered daily and fertilized (Miracle-Gro, water-soluble, all-purpose plant food) weekly.

Tomato was harvested after last injury evaluation (4-weeks after tomato progeny emergence). The harvested tomato fruits (F1 progeny) from dicamba application at 1/16 X, 1/32 X, 1/64 X, and 1/128 X and from untreated check (the tomato progeny or F1) were harvested and planted on May 4 which the seedlings were emerged on May 11. The tomato seedlings (F1 = progeny) were rated (evaluated) one-, two-, three-, and 4-weeks after emergence for any injury or dicamba symptomology.

2.2 Year Two

In year two study, tomato (cherry tomato) seeds were planted in the small pots (6.4 × 6.4 × 7.6 cm) containing putting-mix on September 28, 2021. Tomatoes were emerged on October 2. Tomato seedlings were transplanted in a bigger pot (10.2 × 10.2 × 12 cm) on October 18. Tomato plants were watered and fertilized as needed.

Harvested tomato fruits from dicamba application at 1/16 X, 1/32 X, 1/64 X, and 1/128 X and from untreated check (the tomato progeny or F1) were harvested and planted on January 24, 2022 which the seedlings were emerged on February 1 and transplanted in a bigger pots on February 04. The tomato seedlings (F1 = progeny) were rated (evaluated) one-, two-, three-, and 4-weeks after emergence for any injury or dicamba symptomology.

Tomato injury data is presented for 1-, 2-, and 3-weeks after applications (WAA) of dicamba at 1/16 X, 1/32 X, 1/64 X, and 1/128 X rates at tomato vegetative, flowering, and fruiting stages. Year was significant different for tomato injury rating at 1 WAA and 3 WAA; therefore the data was presented by year. Year was not significant different for tomato injury rating at 2 WAA; therefore the data was combined over years. Data was analyzed with JAMP Pro 13.

Tomato injury from simulated drift rate of dicamba at 1/16 X, 1/32 X, 1/64 X, and 1/128 X was evaluated weekly from 1 to 3 weeks after application (WAA). Application equipment was a CO₂ pressurized backpack sprayer 187 L/ha with a TTI (Turbo Teejet Induction) 11002 nozzles.

Furthermore, in cases where fruit samples were available for analysis, each dicamba treatment was analyzed using high-performance liquid chromatography (HPLC) to identify potential dicamba compounds. The detection of dicamba within the fruit was carried out using an Agilent 1100 series HPLC system (Agilent, Santa Clara, CA). The data processing was performed using the Agilent Chemstation A.10.02 software, which incorporated a spectral module (Agilent Technologies Inc., Wilmington, DE, USA). The separation of compounds took place on an Alltech Adsorbosphere reverse phase C18 column (150 mm × 4.6 mm, Dr. A. Maisch High-Performance LC GmbH, Germany) with a particle size of 3 µm. A wash was included in the 5 µL injection volume. The mobile phase consisted of a mixture of water (59.8%), acetic acid (0.2%), and acetonitrile (40%). The flow rate was set at 0.5 mL/min, with a stop time of 10 minutes and a post time of 2 minutes. The temperature was maintained at 25 °C, and detection was carried out using a diode-array detector (DAD) at 280 nm.

The collected data underwent an analysis of variance, with treatments and runs serving as classification variables. This analysis was conducted using JMP Pro 13 (SAS Institute Inc., SAS Campus Drive, Cary, NC 27513, USA). Subsequently, differentiation of mean values was accomplished utilizing the Fisher's protected LSD test, employing a significance level of ≤ 0.05 .

3. Results and Discussion

3.1 Year One

One WAA of dicamba, tomato plants at the vegetative stage exhibited the highest sensitivity compared to the flowering and fruiting stages (Figure 1). Tomato injury (Leaf malformation, leaf twisting, cupping, stunting, and crinkling) caused by a simulated drift rate of 1/16X dicamba was measured at 67.5%, 36.3%, and 40% at the vegetative, flowering, and fruiting stages, respectively. Even at a lower simulated drift rate of 1/64X dicamba, tomato injury reached 47.5%, 6.3%, and 5% at the vegetative, flowering, and fruiting stages, respectively. The severity of tomato injury increased as simulated dicamba rates increased.

At three WAA of dicamba, tomato plants at the vegetative stage still exhibited the highest sensitivity compared to the flowering and fruiting stages (Figure 2). The highest simulated dicamba rate (1/16X) resulted in tomato injury of 78.8%, 32.1%, and 40% at the vegetative, flowering, and fruiting stages, respectively. The lowest simulated dicamba rate (1/128X) caused tomato injury of 23.8%, 4.6%, and 5% at the vegetative, flowering, and fruiting stages, respectively.

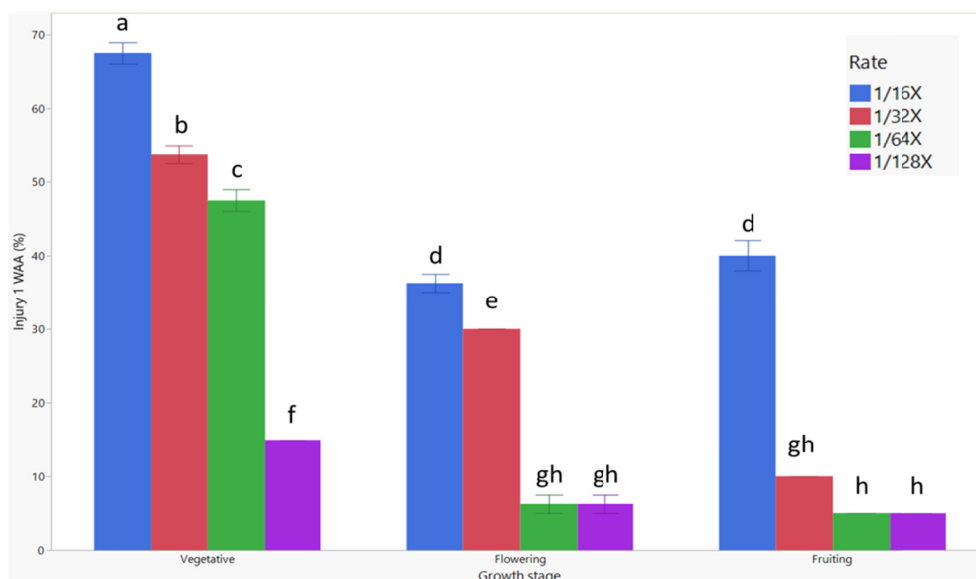


Figure 1. Tomato injury from simulated rate of dicamba at 1/16 X, 1/32 X, 1/64 X, and 1/128 X at vegetative, flowering, and fruiting stages of tomato one-week after application in year 1. Treatments associated with the same letter are not significantly different ($\alpha = 0.05$)

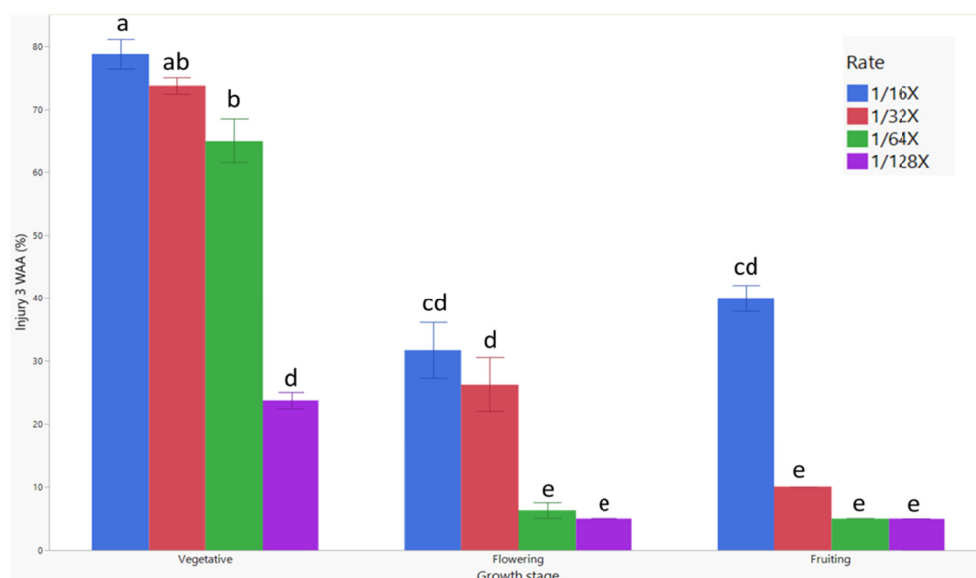


Figure 2. Tomato injury from simulated rate of dicamba at 1/16 X, 1/32 X, 1/64 X, and 1/128 X at vegetative, flowering, and fruiting stages of tomato three-weeks after application in year 1. Treatments associated with the same letter are not significantly different ($\alpha = 0.05$)

3.2 Year Two

One WAA of dicamba, tomato at vegetative stage was most sensitive compared to the flowering or fruiting stage in second year as it did in first year. Tomato injury for simulated drift rate of dicamba at 1/16 X was 75, 35, and 21.3% at vegetative, flowering, and fruiting stage of tomato, respectively. Even dicamba simulated drift rate of 1/64 X caused 57.5, 12.5, and 7.3% tomato injury at vegetative, flowering, and fruiting stage, respectively. (Figure3). Tomato injury increased as simulated drift rate of dicamba increased from 1/128 X to 1/16 X.

Three WAA of dicamba, still tomato at vegetive stage showed most sensitivity compared to the flowering or fruiting stage. The highest simulated rate of dicamba (1/16 X) caused 75, 37.5, and 20% tomato injury at vegetative, flowering, and fruiting stage of tomato, respectively. The lowest simulated rate of dicamba (1/128 X)

caused 35, 2.8, and 5% tomato injury at vegetative, flowering, and fruiting stage of tomato, respectively (Figure 4).

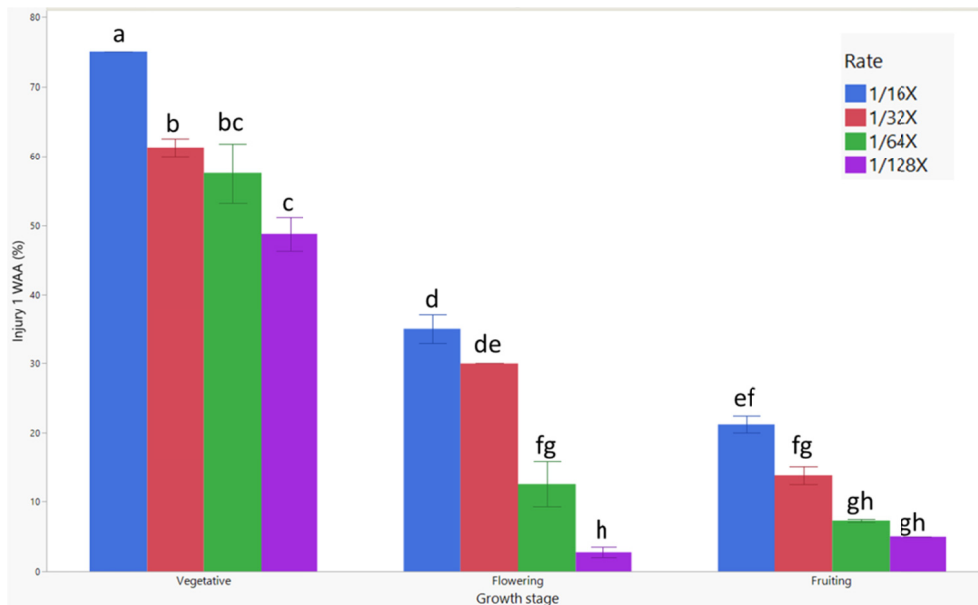


Figure 3. Tomato injury from simulated rate of dicamba at 1/16 X, 1/32 X, 1/64 X, and 1/128 X at vegetative, flowering, and fruiting stages of tomato one-week after application in year 2. Treatments associated with the same letter are not significantly different ($\alpha = 0.05$)

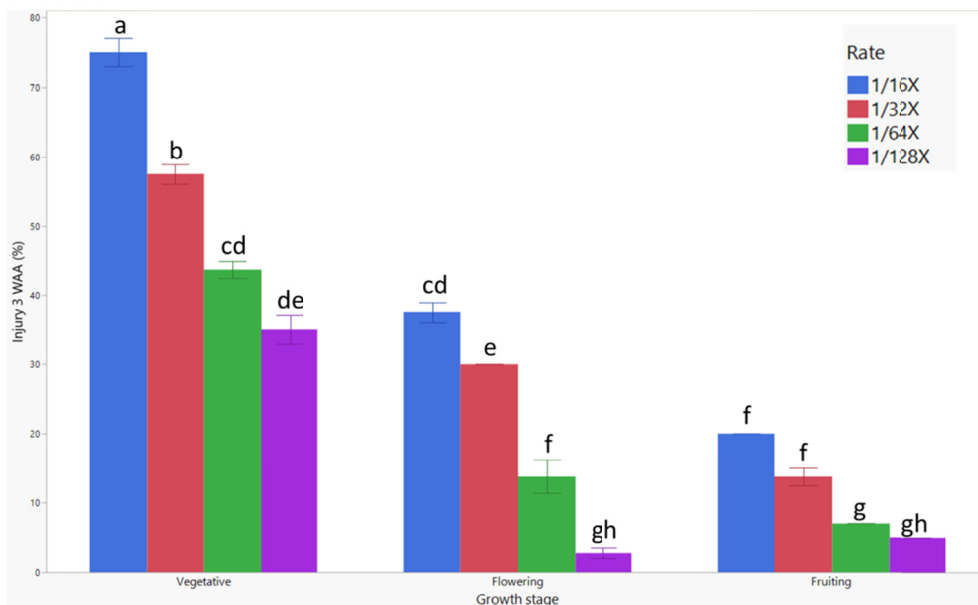


Figure 4. Tomato injury from simulated rate of dicamba at 1/16 X, 1/32 X, 1/64 X, and 1/128 X at vegetative, flowering, and fruiting stages of tomato three-weeks after application in year 2. Treatments associated with the same letter are not significantly different ($\alpha = 0.05$)

3.3 Year Combined for 2 WAA

Tomato injury rating of 2 WAA of simulated rate of dicamba was combined over the years since year was not significant different. Two WAA of simulated drift rate of dicamba, still tomato at vegetive stage showed most sensitivity compared to the flowering or fruiting stage. The highest simulated rate of dicamba (1/16 X) caused

78.1, 35.6, and 31.3% tomato injury at vegetative, flowering, and fruiting stage of tomato, respectively. The lowest simulated rate of dicamba (1/128 X) caused 34.4, 3.9, and 4.3% tomato injury at vegetative, flowering, and fruiting stage of tomato, respectively (Figure 5).

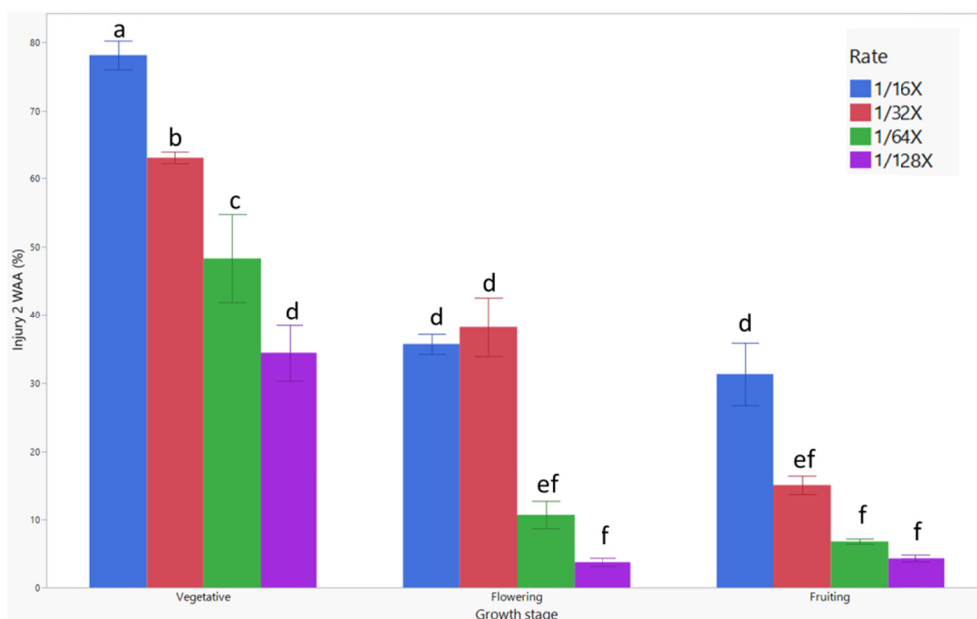


Figure 5. Tomato injury from simulated rate of dicamba at 1/16 X, 1/32 X, 1/64 X, and 1/128 X at vegetative, flowering, and fruiting stages of tomato two-weeks after application averaged over years. Treatments associated with the same letter are not significantly different ($\alpha = 0.05$)

3.4 Tomato Progeny (F1) Injury in Year One

The seedlings at the vegetative stage (application A = 5- to 6-leaf tomato with 23- to 25-cm in height) sprayed on February 24, 2021 did not produce any fruit except the untreated check. However, tomato at flowering (application B) and tomato at fruiting stage (application C), which were sprayed with dicamba on March 23 and April 21, produced fruits.

Harvested tomato fruits (May 4, 2021) from dicamba application at 1/16 X, 1/32 X, 1/64 X, and 1/128 X and from untreated check (the tomato progeny or F1) were planted on May 4, and seedlings emerged on May 11. The tomato seedlings (F1 = progeny) were evaluated one-, two-, three-, and four-weeks after emergence for any injury or dicamba symptomology. The tomato progeny (F1) was evaluated four times (every week) and did not show any visual dicamba symptomology (Figure 10). Some fruit sample of each dicamba treatments which produce fruit was sent to the lab for HPLC (High-performance liquid chromatography) analysis to detect any dicamba compound in the fruit. The results of the HPLC analysis indicated that none of the fruit samples showed any levels of dicamba. Samples were taken from upper, middle, and lower fruits from each treatment and did not find any dicamba in them.

3.5 Tomato Progeny (F1) Injury in Year Two

Tomato seedlings at vegetative stage (application A = 5- to 6-leaf tomato with 23- to 25-cm in height) which sprayed on October 28 did not provide any fruit (most plants) and some died except the untreated check. However, tomato at flowering (application B) and tomato at fruiting stage (application C) which sprayed with dicamba on November 16 and December 10 provided fruits.

Harvested tomato fruits from different dicamba treatment rates (1/16X, 1/32X, 1/64X, and 1/128X) and untreated check (tomato progeny or F1) were planted, and subsequent seedlings were evaluated for dicamba injury at one-, two-, three-, and four-week intervals after emergence. Visual examination of the tomato progeny (F1) at the four-week evaluation did not reveal any dicamba symptomology. Additionally, fruit samples from each dicamba treatment that produced fruit were analyzed using High-performance Liquid Chromatography (HPLC) to detect the presence of dicamba compounds. The HPLC analysis results demonstrated the absence of

dicamba at detectable levels in all fruit samples. Samples were collected from different parts (upper, middle, and lower) of the fruits across each treatment, further confirming the absence of dicamba residue.

The results of this study indicate that the sensitivity of tomato plants to dicamba application varied depending on the growth stage. In the first year, tomato plants at the vegetative stage (Figures 6, 7, 8, and 9) showed the highest sensitivity to dicamba, resulting in significant injury compared to the flowering and fruiting stages. The severity of tomato injury increased as the simulated drift rate of dicamba increased from 1/128X to 1/16X. Similar trends were observed in the second year, with tomato plants at the vegetative stage being the most sensitive to dicamba. These findings suggest that the vegetative stage of tomato plants is particularly vulnerable to dicamba exposure. This is in contrast to Griffin et al. (2013) who found soybean injury increased with higher dicamba rates and was more pronounced when applied at the flowering stage (R1). Yield reductions were observed, with flowering soybean being more sensitive to dicamba compared to vegetative exposure. Similarly, field studies assessed the impact of sublethal glyphosate and dicamba doses on flowering loss and marketable yield in processing tomatoes (Kruger et al., 2012). Four tomato lines were studied, and both herbicides caused greater yield losses when applied at the early bloom stage compared to the vegetative stage. Dicamba exhibited higher sensitivity in tomato cultivars than glyphosate, indicating potential yield implications from drift, particularly during the flowering period.

When evaluating the tomato progeny (F1) in both years, no visual dicamba symptomatology was observed, indicating that the use of low rates of dicamba did not result in noticeable injury to the offspring plants. On the contrary, Jones et al. (2019) observed auxin-like symptoms, such as leaf cupping in offspring soybean plants, more prominently in later reproductive stages. Parent soybean plants treated with dicamba during the R5 growth stage showed significant correlations with offspring emergence, vigor, injury, and percent of injured plants, suggesting that damage to soybean plants during reproductive stages can adversely affect their offspring. In our study, HPLC analysis of fruit samples from the dicamba-treated plants did not detect any dicamba compounds, suggesting that the fruit did not contain detectable levels of the herbicide. This finding is crucial for ensuring the safety and quality of tomato produce. The combined analysis of two weeks after application revealed that tomato plants at the vegetative stage remained the most sensitive to dicamba, consistent with the individual year findings. These results emphasize the importance of considering the growth stage of tomato plants when applying dicamba, as early exposure during the vegetative stage can lead to more significant injury.

Although the results from this study demonstrate the absence of dicamba symptoms and residue in the tomato progeny and fruit samples, it is important to note that these findings are based on one simulated drift event. In real-world scenarios where multiple dicamba drift events may occur, the potential damage to tomato crops could be more severe. A study investigated the impact of dicamba-based herbicides, including Clarity®, Engenia®, and XtendiMax®, on grape and tomato plants in 2016 and 2017 (Knezevic et al., 2018). The research found that even at micro-rates, these dicamba formulations negatively affected plant growth, with as little as 2% of the label rate causing significant injury and reduction in vine length or plant height. While grape appeared more sensitive to XtendiMax® compared to Clarity® and Engenia®, tomato sensitivity did not significantly differ among the three products, highlighting the need to prevent dicamba drift onto these crops despite advancements in reducing volatility. Therefore, caution should be exercised by applicators when using dicamba near sensitive crops like tomato, especially in cases of repeated exposure.



Figure 6. Tomato injury symptom (at vegetative stage) from simulated drift rate of dicamba at 1/16 X



Figure 7. Tomato injury symptom (at vegetative stage) from simulated drift rate of dicamba at 1/32 X



Figure 8. Tomato injury symptom (at vegetative stage) from simulated drift rate of dicamba at 1/64 X



Figure 9. Tomato injury symptom (at vegetative stage) from simulated drift rate of dicamba at 1/128 X



Figure 10. Tomato progeny (F1) show no injury or dicamba symptomology from dicamba application at 1/16 X, 1/32 X, 1/64 X, and 1/128 X at flowering and fruiting stages

4. Conclusions

In conclusion, this study found that tomato plants at the vegetative stage were more sensitive to low-rate dicamba application compared to the flowering or fruiting stages. However, there was no significant difference in tomato injury between the flowering and fruiting stages. Tomato injury increased as the simulated drift rate of dicamba increased. Tomato seedlings at the vegetative stage did not produce fruit, but those sprayed with dicamba during the flowering and fruiting stages yielded fruits. The tomato progeny did not exhibit visual dicamba symptoms, and HPLC analysis detected no dicamba residue in the fruit samples. These results are based on a single simulated drift event, and caution should be exercised when applying dicamba near sensitive crops like tomato, especially in scenarios involving multiple drift events. Further research is needed to explore the long-term effects of repeated dicamba exposure and develop effective mitigation strategies for protecting sensitive crops.

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Acknowledgments

This publication is a contribution of the Mississippi Agricultural and Forestry Experiment Station. This material is based upon work that is supported by the Mississippi Department of Agriculture and Commerce (MDAC) and the National Institute of Food and Agriculture, U.S. Department of Agriculture, Hatch project under accession number 151510.

Authors Contributions

Not applicable.

Funding

Not applicable.

Competing Interests

The authors declare no conflict of interests.

Informed Consent

Obtained.

Ethics Approval

The Publication Ethics Committee of the Canadian Center of Science and Education.

The journal's policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).

Provenance and Peer Review

Not commissioned; externally double-blind peer reviewed.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data Sharing Statement

No additional data are available.

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