agriculture

Article

Sensitivity and Recovery of Tomato Cultivars Following Simulated Drift of Dicamba or 2,4-D

Michele R. Warmund *, Mark R. Ellersieck and Reid J. Smeda

Division of Plant Sciences and Technology, University of Missouri, Columbia, MO 65211, USA
* Correspondence: warmundm@missouri.edu

Abstract: Tomato (*Solanum lycopersicum* L.) plants are commonly injured by the off-target movement of synthetic auxin herbicides. A greenhouse and a field trial were conducted to determine the relative tolerance of eight fresh market tomato cultivars to drift-simulating rates of dicamba or 2,4-D. Tomato cultivars included 'BHN 589', 'Celebrity', 'Florida 91', 'Mountain Merit', 'Primo Red', 'Red Deuce', 'Red Morning', and 'Skyway'. Dicamba (3,6-dichloro-methoxybenzoic acid) and 2,4-D (2,4-dichlorophenoxyacetic acid) were applied at 2.8 g ae ha^{-1} and 5.3 g ae ha^{-1}-D, respectively. By 14 weeks after treatment (WAT), herbicide-treated plants of each cultivar produced less total and marketable yield than their respective nontreated control in the greenhouse trial. For most cultivars, dicamba-treated plants had less marketable yield than 2,4-D-treated plants in the greenhouse. Herbicide treatments also reduced total and marketable yields of each cultivar when compared with their control in the field study at 14 WAT, except for 'Mountain Merit'. The severity of yield loss from herbicide treatments was cultivar-dependent. Field-grown 'Skyway' plants treated with dicamba produced the lowest marketable yield. In contrast, herbicide-treated plants of 'Florida 91' produced high marketable yields in the field, but 'Red Deuce' plants receiving 2,4-D were also highly productive. Herbicide residue in fruit sampled the third week of the harvest was nondetectable. Because the type of auxin herbicide drift is often unanticipated, 'Florida 91' may be the preferred cultivar for cultivation among those tested to maximize tomato production in the field.

Keywords: *Solanum lycopersicum*; auxin herbicides; flower abortion; sublethal rate; tomato fruit yield

Citation: Warmund, M.R.; Ellersieck, M.R.; Smeda, R.J. Sensitivity and Recovery of Tomato Cultivars Following Simulated Drift of Dicamba or 2,4-D. *Agriculture* **2022**, *12*, 1489. https://doi.org/10.3390/agriculture12091489

Academic Editors: Fernanda Fidalgo and Conceição Santos

Received: 11 August 2022
Accepted: 14 September 2022
Published: 17 September 2022

1. Introduction

Tomatoes are grown in every region of the United States (U.S.) for the fresh market and for processing, with a production value of approximately USD 1 billion in 2020 [1]. In the midwestern U.S., tomatoes are often grown commercially near soybean fields where auxin-type herbicides, such as dicamba or 2,4-D, are applied for postemergence weed control. Herbicide formulations, including the diglycolamine salt of dicamba and choline salt of 2,4-D, were developed to decrease their volatility and drift in the environment [2]. Moreover, improvements in equipment technology and the addition of drift reduction adjuvants have reduced particle drift of auxin-type herbicides [3]. However, the off-target movement of spray material is also affected by ambient temperature, relative humidity, vapor pressure, and wind speed [4–9]. Despite industry efforts to reformulate these herbicides and reduce their off-target movement, injury has been reported on sensitive plants at low rates [10–13]. U.S. offices that regulate pesticides received over 2200 complaints of suspected herbicide injury on multiple crops in 2017 [14]. More than 1000 complaints, mainly for dicamba injury, were filed in Missouri alone from 2017 to 2019, including 7650 ha of tomato [15].

Typical injury symptoms from drift of dicamba or 2,4-D on tomato include epinasty, foliar distortion, split stems with protruding root initials, and malformed fruit with delayed maturity [16–19]. The severity of tomato injury is dependent upon the herbicide dose and the plant growth stage at exposure [20]. Dicamba applied at 2.4 to 13.3 g ae ha^{-1} caused as much as 5 to 25% estimated flower loss, respectively, when tomato plants were treated at an early non-flowering stage [12]. However, lower rates of dicamba at 1.5 to 6.4 g ae ha^{-1} resulted in similar estimations of flower abortion when plant exposure occurred at early bloom. Applications of 2,4-D at rates ranging from 1.8 to 2.1 g ae ha^{-1} at first bloom caused malformed fruit and reduced total yield [18,19]. Moreover, applications of 2,4-D at first bloom caused greater crop loss and delays in fruit maturity than those applied at a later stage when much of the crop was set [19]. 'Big Beef' tomato plants treated before flowering with dicamba at 2.8 g ae ha^{-1} or 2,4-D at 5.3 g ae ha^{-1} had similar total fruit weights at 12 WAT [21]. In the same study, dicamba-treated 'Florida 91' plants produced less total fruit weight than 2,4-D-treated plants at 12 WAT. The rates of herbicides in the aforementioned reports are comparable with those occurring from typical drift events [12,18–21].

The severity of 2,4-D injury is also genotype-dependent. In an early study, 'Roma', 'Heinz 1439', 'Moreton Hybrid', 'Glamour', and 'Galaxy' tomato had the highest resistance to 2,4-D injury when 50 genotypes were treated at early stages of flowering to fruit set [22]. More recently, three wild tomato accessions (TOM199, TOM198, and TOM300) exhibited less injury to dicamba and 2,4-D at 3 and 11 g ae ha^{-1}, respectively, than commercially-available 'Money Maker' and 'Better Boy' [23]. However, wild tomato accessions were killed at rates of dicamba ranging from 140 to 280 g ae ha^{-1} [24].

While there has been progress in developing an auxin herbicide-tolerant tomato, commercial cultivars suitable for the fresh market have yet to be released. In the meantime, mitigation strategies to limit crop loss of herbicide-sensitive plants are needed. Moreover, dicamba and 2,4-D drift injury on plants at a pre-bloom growth stage has not been widely assessed on tomato cultivars commonly grown by producers. Thus, this study was conducted to evaluate the sensitivity of eight commercially-available fresh market tomato cultivars to dicamba and 2,4-D at a drift-simulating rate of application and to determine if herbicide residue was detectable in fruit produced following herbicide treatment.

2. Materials and Methods

2.1. Greenhouse Trial

This trial was conducted at the University of Missouri, Columbia, MO (38°94′03.25″ N latitude, 92°31′80.9″ W longitude) in 2021. Commonly grown, fresh market, determinate-type tomato cultivars were used for this study, except for 'Celebrity', which is a semi-determinate type. 'BH 589', 'Celebrity', 'Florida 91', 'Mountain Merit', 'Primo Red', 'Red Deuce', 'Red Morning', and 'Skyway' tomato seeds (Tomato Growers Supply, Fort Myers, FL, USA) were planted a growing medium of sphagnum moss, vermiculite, and perlite (ProMix BX; Premier Tech Horticulture, Québec, QC, Canada) in shallow containers (20 × 27 cm) in May 2021 for germination in a glass greenhouse. The average air temperatures in the glasshouse during the study were 26 °C day/22 °C night cycle. Because the greenhouse had been treated with whitewash (Redusol; Mardenkro, Baarle-Nassau, The Netherlands), high-pressure sodium lighting (Sunlight Supply, Inc., Vancouver, WA, USA) delivering a photosynthetic photon flux density of 90 μmol m^{-2} s^{-1} at the plant canopy for a 14 h constant photoperiod was used. Tomato seedlings (4 cm-tall) were transplanted into 0.5-L pots in the same growing medium. Throughout the study, plants were watered by hand daily and grown in the greenhouse under the environmental conditions described above.

Tomato plants had 7 or 8 leaves per plant and had no apparent flower buds when dicamba (Xtendimax; Bayer CropScience, St. Louis, MO, USA) at 2.8 g ae ha^{-1} and 2,4-D (Enlist One; Dow AgroSciences, Indianapolis, IN, USA) at 5.3 g ae ha^{-1} were applied on 15 June 2021. These rates represent 1/200 of the rate of application listed on the product label (560 g ae ha^{-1} dicamba and 1060 g ae ha^{-1} 2,4-D) for transgenic soybean. Moreover, nontreated control plants were included as a standard of comparison. A backpack sprayer with 8002 flat fan nozzles (TeeJet Technologies, Urbandale, IA, USA), which was pressurized at 193 kPa, was used outdoors to apply herbicides 43 cm above the plant foliage at 140 L ha^{-1}. After the spray solution on the tomato foliage dried, plants were isolated by treatment in three different greenhouses (15 m-apart) for seven days to avoid herbicide movement and cross-contamination among plants. Next, plants were shifted into 8.5-L pots, placed in a whitewashed greenhouse, and maintained under the conditions described above. On 1 July 2021, a 2.4 m-long bamboo stake (A.M. Leonard, Piqua, OH, USA) was inserted into each container, and tomato plants were tied to each stake weekly. Plants were hand-watered daily to field capacity and fertilized with a 15 g L^{-1} solution of calcium nitrate 15N-0P-0K (15-0-0, YaraLiva, Greenway Biotech Inc., Santa Fe Springs, CA, USA) using 800 mL per container alternating weekly with a 15 g L^{-1} solution of 4N-7.9P-31.5K (4-18-38, Masterblend, Masterblend International, Morris, IL, USA). Plants were not pruned during the study period. This experiment was a randomized complete block design in a factorial arrangement (8 cultivars $\times$ 3 herbicide treatments) with five single-plant replications of each treatment combination.

Plant height was recorded immediately before treatment. At 4 WAT (weeks after treatment), herbicide injury was estimated using a rating scale of 0 (no foliar and stem injury) to 10 (100% foliar and stem injury). Injury ratings, such as 1, 3, 5, and 9, were used to indicate that 10, 30, 50, 70, and 90%, respectively, of the foliage exhibited epinasty. Moreover, increases in plant height were recorded at this time. Tomatoes were harvested at the light red to the red stage at three-day intervals from 4 August to 21 September 2021. At each harvest, fruit $\geq$ 54 mm-diameter and free of cracking or any other visible defect were recorded as marketable yield, according to U.S. Department of Agriculture standards for grade no. 1 fresh tomatoes [25]. Fruit < 54 mm-diameter or with defects were considered nonmarketable yield. Fresh weights of both types of fruit from each plant were recorded at each harvest date, and the total yield was calculated as the sum of marketable and nonmarketable fruit. At 14 WAT, the number of live and dead reproductive organs (flower buds, blossoms, and all fruit) were recorded. Withered flowers that failed to set fruit were considered aborted.

Data were subjected to analysis of variance (ANOVA) using PROC GLIMMIX procedure in SAS (version 9.4; SAS Institute, Cary, NC, USA). Injury ratings for plants treated with an herbicide were rank-transformed. However, back-transformed injury ratings are presented since the means were similar. The odds (i.e., probability) of fruit set as a proportion of the total number of flowers were calculated, and the GLMMIX procedure with a link = logit function for a binomial distribution was used for data analysis. Odds were calculated from the antilog of the logit value and back-transformed [% nonmarketable fruit set = odds/(1 + odds)] for the presentation of the data. Moreover, the odds of nonmarketable fruit as a proportion of total fruit yield were calculated and analyzed using the same procedure. Means were separated by Fisher's protected least significant difference (LSD) test, $p \leq 0.05$.

2.2. Field Trial

This trial was conducted at the Horticulture and Agroforestry Research Center, New Franklin, MO, USA (39°00′34.8″ N latitude, 92°44′10.79″ longitude) in 2021. The soil type was a Menfro silt loam (fine-silty, mixed, superactive, mesic typic hapludalfs) with 2.7% organic matter and a pH of 6.5. One week before planting, urea 46N-0P-0K (46-0-0, YaraVera, Greenway Biotech Inc., Santa Fe Springs, CA, USA) at 48 kg ha^{-1} and 6N-10.5P-19.9K (6N-24P-24K, Andersons, Maumee, OH, USA) fertilizers at 186 kg ha^{-1} were incorporated into the soil at a 20 cm depth, using a roto-tiller. White surface polyethylene mulch (1.5 m wide, Growers Solution, Cookeville, TN, USA) and irrigation tape with 30-cm emitter spacing and 1.14 L h^{-1} flow per emitter (Hardie Irrigation, Sanford, FL, USA) were applied to the test plot by machine.

Tomato seeds were sown in trays, and plants were grown in containers and treated with herbicides as described above before field transplanting on 16 June 2021. Plants were spaced 0.9 m within a row and 2.4 m between rows. Plants were supported using a stake-and-weave system with metal posts (2.1 m height) and polyethylene string [26]. Plants were irrigated as needed and fertilized weekly, as described above. Herbicide injury was assessed at 4 WAT, and fruit was harvested, sorted, and weighed by marketable and unmarketable fruit as described above until 14 WAT. Three fruit per treatment in each replication were sampled from 23 to 26 August (during the third week of harvest) for dicamba or 2,4-D residue analyses. Samples were shipped overnight to South Dakota Agricultural Laboratories (Brookings, SD, USA). Herbicide analytes were extracted with dichloromethane for measurement by gas chromatography-mass spectrometry/mass spectrometry, using the method described by Wen [27] with a quantification limit of 0.005 ug g^{-1}. The experiment was a randomized complete block design in a factorial arrangement (8 cultivars × 3 herbicide treatments), using four replications with three plants for each replicate of each treatment combination. Data for herbicide injury ratings, total yield, and unmarketable fruit were analyzed as described above.

3. Results

3.1. Greenhouse Trial

Both herbicides caused cupping, distortion, and undersized foliage, as well as stem epinasty and stunting on all cultivars. Moreover, the tips of leaflets on all cultivars treated with either herbicide were more acutely tapered than nontreated control leaflets (Figures 1 and 2). The apex of the primary stem of plants treated with dicamba often ceased elongation and failed to produce viable flowers (Figure 3). At 4 WAT, the main effect of treatment was significant ($p < 0.0001$) for herbicide injury ratings. Dicamba-treated plants had a higher injury rating (4.3) than 2,4-D-treated plants (2.9). The main effect of cultivar and the interaction of cultivar × treatment were not significant ($p = 0.8341$ and $p = 0.1331$, respectively).

Figure 1. Foliar epinasty and acutely tapered tips of leaflets from plants treated with 2,4-D at 5.3 g ae ha^{-1} at four weeks after treatment: (**a**) 'BHN 589', (**b**) 'Celebrity', (**c**) 'Florida 91', (**d**) 'Mountain Merit', (**e**) 'Primo Red', (**f**) 'Red Deuce', (**g**) 'Red Morning', and (**h**) 'Skyway'. Plants treated with dicamba at 2.8 g ae ha^{-1} also expressed similar injury symptoms, with sharply tapered leaflet tips at the same time.

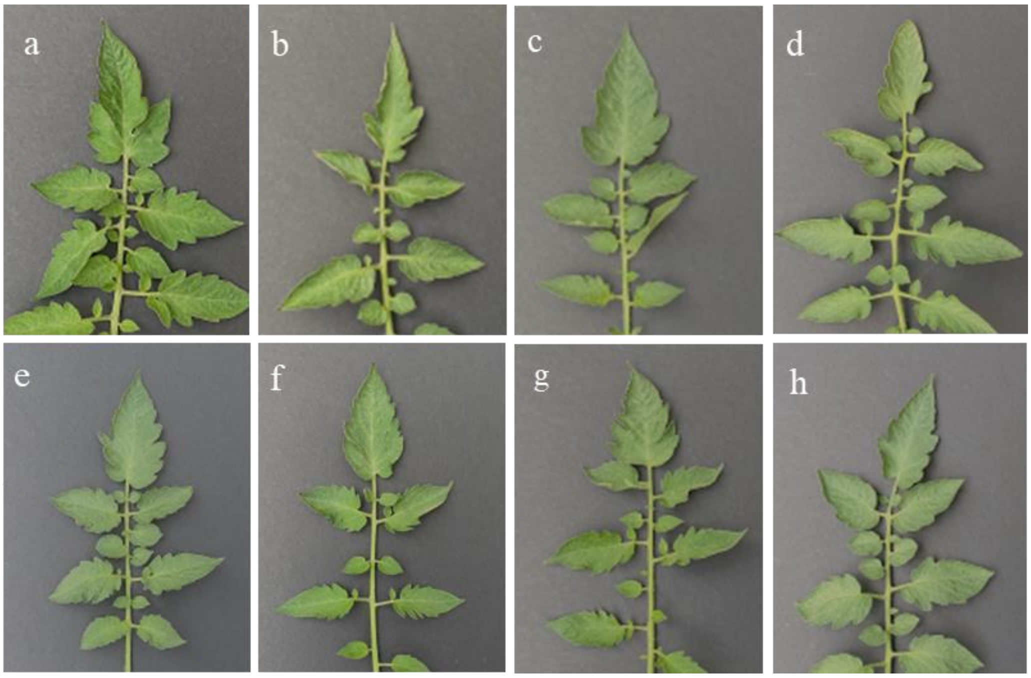

Figure 2. Nontreated control leaflets from (**a**) 'BHN 589', (**b**) 'Celebrity', (**c**) 'Florida 91', (**d**) 'Mountain Merit', (**e**) 'Primo Red', (**f**) 'Red Deuce', (**g**) 'Red Morning', and (**h**) 'Skyway' plants at four weeks after treatment.

Figure 3. The stunted main stem and aborted floral organs on a 'Skyway' plant treated with dicamba at 2.8 g ae ha^{-1} at four weeks after treatment.

The i©nteraction of cultivar and treatment was significant for plant height at 4 WAT (Table 1). For each cultivar, nontreated controls had a greater increase in plant height compared with herbicide-treated plants, with dicamba causing more plant stunting than 2,4-D for every cultivar. For the nontreated controls, 'Mountain Merit', 'Red Deuce', and 'Skyway' had the greatest increase in plant height. However, for dicamba-treated plants, 'Primo Red' and 'Skyway' had a greater increase in height than all other cultivars, except for 'Mountain Merit'. For 2,4-D treatments, 'Mountain Merit' and 'Primo Red' had the greatest increase in plant height. 'BHN 589' plants always produced the least new growth within each treatment.

Table 1. Plant height of tomato cultivars treated with an application of dicamba or 2,4-D and grown in a greenhouse at 4 WAT [1].

Cultivar	Plant Height (cm)		
	Control	Dicamba	2,4-D
BHN 589	55.0 Ea	42.2 Dc	47.8 Eb
Celebrity	64.4 Ba	53.2 Bc	59.2 Db
Florida 91	58.2 Da	45.6 Cc	55.6 Cb
Mountain Merit	67.2 Aa	54.4 ABc	63.4 Ab
Primo Red	65.0 Ba	55.0 Ac	63.0 Ab
Red Deuce	67.6 Aa	53.6 Bc	61.8 Bb
Red Morning	59.6 Ca	46.8 Cc	55.0 Cb
Skyway	66.4 Aa	55.6 Ac	61.8 Bb
Significance		*p*-value	
Cultivar (C)		<0.0001	
Treatment (T)		<0.0001	
C × T		<0.0001	

[1] Means represent the increase in plant height that occurred from the initial measurement recorded immediately before treatment to 4 WAT (weeks after treatment). Dicamba and 2,4-D were applied at a drift-simulating rate of 2.8 g ae ha^{-1} and 5.3 g ae ha^{-1}, respectively. Values represent the mean of 5 replications of each treatment. Treatment means within a column followed by the same uppercase letters and cultivar means within a row followed by the same lowercase letters are not significantly different, according to Fisher's protected LSD test ($p \leq 0.05$).

The main effect of treatment was significant for percent fruit set ($p = 0.0244$) at 14 WAT. Control plants had a higher percent fruit set (48%) than those treated with dicamba (35%) or 2,4-D (38%). The main effect of cultivar and the interaction of cultivar and treatment for percent fruit set were not significant ($p = 0.0630$ and $p = 0.1814$, respectively).

The interaction of cultivar by treatment was significant for all flower and fruit yield variables shown in Tables 2 and 3. For each cultivar, herbicide-treated plants had more aborted flowers than the control, except for 'Skyway' plants treated with 2,4-D (Table 2). Further, dicamba caused more flower abortion than 2,4-D for each cultivar, except for 'Celebrity', 'Primo Red', and 'Red Deuce'. For 2,4-D-treated plants, 'Florida 91' and 'Mountain Merit' had relatively low flower abortion compared with other cultivars (i.e., 13 to 36% less).

Table 2. Numbers of aborted flowers and live reproductive organs per plant of tomato cultivars treated with an application of dicamba or 2,4-D and grown in a greenhouse [1].

Cultivar	Number of Aborted Flowers/Plant			Number of Live Reproductive Organs/Plant		
	Control	Dicamba	2,4-D	Control	Dicamba	2,4-D
BHN 589	208 Ac	306 Ba	259 Ab	422 Ab	471 Ba	421 Ab
Celebrity	201 Ab	261 Ca	240 ABa	350 Bb	410 Ca	360 Bb
Florida 91	117 Dc	228 Da	169 Db	260 Db	349 Ea	270 Eb
Mountain Merit	105 Dc	259 Ca	167 Db	222 Ec	406 Ca	267 Eb
Primo Red	121 CDc	196 Eb	232 ABa	224 Ec	323 Fa	265 Eb
Red Deuce	136 Cb	202 Ea	195 CDa	270 Dc	313 Fa	252 Fb
Red Morning	161 Bc	296 Ba	216 BCb	331 Cb	385 Da	328 Db
Skyway	208 Ab	350 Aa	223 BCb	354 Bc	494 Aa	340 Cc
Significance			***p*-value**			
Cultivar (C)		<0.0001			<0.0001	
Treatment (T)		<0.0001			<0.0001	
C × T		<0.0001			<0.0001	

[1] Dicamba and 2,4-D were applied at a drift-simulating rate of 2.8 g ae ha^{-1} and 5.3 g ae ha^{-1}, respectively. WAT = weeks after treatment. Values represent the mean of 5 replications of each treatment. Treatment means within a column followed by the same uppercase letters and cultivar means within a row followed by the same lowercase letters are not significantly different, according to Fisher's protected LSD test ($p \leq 0.05$).

Table 3. Total fruit yield, marketable yield, and percent non-marketable yield of tomato cultivars treated with an application of dicamba or 2,4-D and grown in a greenhouse [1].

Cultivar	Total Yield/Plant (g)			Marketable Yield/Plant (g)			Nonmarketable Yield/Plant (%) [2]		
	Control	Dicamba	2,4-D	Control	Dicamba	2,4-D	Control	Dicamba	2,4-D
BHN 589	1432 Ga	913 Gc	1006 Hb	1352 Ga	785 Hc	973 Hb	5.6 Cb	14.1 Ca	3.2 Fc
Celebrity	1418 Ha	1150 Dc	1206 Gb	1249 Ha	996 Dc	1121 Fb	11.9 Aa	12.8 Da	7.0 Eb
Florida 91	2315 Da	1139 Ec	1481 Db	2207 Ca	967 Ec	1350 Db	4.9 Dc	15.1 Ca	8.8 Cb
Mountain Merit	2254 Ea	1118 Fc	1975 Bb	1987 Ea	795 Gc	1662 Cb	11.5 Ac	28.9 Aa	15.8 Bb
Primo Red	2870 Ba	1964 Cc	2136 Ab	2712 Ba	1738 Cc	1967 Ab	5.5 Cc	11.5 Ea	7.9 Db
Red Deuce	3330 Aa	2317 Ac	1890 Cb	3155 Aa	2062 Ab	1768 Bc	5.3 CDc	11.0 Ea	6.4 Eb
Red Morning	2371 Ca	2054 Bb	1412 Ec	2187 Da	1906 Bb	1296 Ec	7.8 Bab	7.2 Fb	8.2 CDa
Skyway	1795 Fa	1115 Fc	1321 Fb	1582 Fa	863 Fc	1079 Gb	11.8 Ac	22.6 Ba	18.3 Ab
Significance					***p*-Value**				
Cultivar (C)		<0.0001			<0.0001			<0.0001	
Treatment (T)		<0.0001			<0.0001			<0.0001	
C × T		<0.0001			<0.0001			<0.0001	

[1] Dicamba and 2,4-D were applied at a drift-simulating rate of 2.8 g ae ha^{-1} and 5.3 g ae ha^{-1}, respectively. WAT = weeks after treatment. Values represent the mean of 5 replications of each treatment. Treatment within a column followed by the same uppercase letters and cultivar means within a row followed by the same lowercase letters are not significantly different, according to Fisher's protected LSD test ($p \leq 0.05$). [2] PROC GLIMMIX using a link = logit function for binomial distributions was used to analyze non-marketable yield as a proportion of total fruit yield. Back transformed data [% non-marketable yield = odds/(1 + odds)] are presented.

By 14 WAT, dicamba-treated plants had more live reproductive organs than 2,4-D-treated or control plants for each cultivar (Table 2). Moreover, 2,4-D-treated plants of all cultivars had similar or higher numbers of reproductive organs compared with their respective nontreated control, except for 'Red Deuce'.

The first date of harvest and subsequent fruit ripening were similar among all treatments (data not shown). For each cultivar, control plants produced the greatest total fruit yield, 2,4-D-treated plants had intermediate yields, and dicamba-treated plants produced the lowest yields, except for 2,4-D-treated 'Red Morning' (Table 3). 'Red Deuce' produced the highest yields among nontreated controls and dicamba treatments, whereas 'Primo Red' produced the highest total yield among 2,4-D-treated cultivars.

Dicamba and 2,4-D reduced marketable yield compared with the respective nontreated control for each cultivar (Table 3). 'Red Deuce' and 'Red Morning' were the only dicamba-treated cultivars that produced more marketable yield than 2,4-D-treated plants of the same cultivar. Among dicamba- and 2,4-D-treated plants, 'Red Deuce' and 'Primo Red' produced the greatest marketable yields, respectively. 'BHN 589' produced the lowest marketable yields among both herbicide treatments.

For the first 2 to 3 weeks of harvest, most nonmarketable fruit were categorized as such due to their small size (<54 mm-diameter). Moreover, 'Primo Red', 'Red Morning', 'Skyway', and 'Celebrity' fruit from herbicide-treated plants were elongated compared with those from nontreated controls. A pronounced tip at the blossom-end of early-harvested 'Primo Red' fruit from dicamba-treated plants was evident (Figure 4). By mid-harvest, misshapen fruit were no longer evident.

Within each cultivar, dicamba-treated plants had higher percentages of nonmarketable yield than nontreated controls, except for 'Celebrity' and 'Red Morning' (Table 3). Dicamba-treated plants also had higher percentages of nonmarketable yield than 2,4-D-treated plants within each cultivar, except for 'Red Morning'. Among dicamba and 2,4-D treatments, 'Mountain Merit' and 'Skyway' had higher percentages of nonmarketable fruit than other cultivars. However, within dicamba-treated plants, 'Red Morning' had the lowest percentage of nonmarketable fruit (7.2%), while within 2,4-D treated plants, 'BHN 589' had the lowest percentage of nonmarketable fruit (3.2%).

Figure 4. Comparison of fruit from the second week of harvest: (**Top**) row: fruit from plants treated with dicamba at 2.8 g ae ha^{-1}. (**Middle**) row: fruit from plants treated with 2,4-D at 5.3 g ae ha^{-1}. (**Bottom**) row: fruit from nontreated control plants.

3.2. Field Trial

At 4 WAT, injury symptoms on field-grown plants appeared slightly less severe than on comparable plants in the greenhouse. For the field trial, only the main effect of treatment was significant for herbicide injury ratings ($p < 0.0001$). Dicamba-treated plants had a higher injury rating (3.8) than 2,4-D-treated plants (2.5).

For each cultivar, herbicide treatments reduced total and marketable fruit yield at 14 WAT compared with the nontreated controls, except for 'Mountain Merit' (Table 4). Moreover, 2,4-D-treated plants produced more total and marketable yield than dicamba-treated plants for each cultivar, except for 'BHN 589', 'Celebrity', 'Florida 91', and 'Mountain Merit'. Within each treatment, 'Florida 91' produced the greatest total yield. 'Florida 91' plants also had greater marketable yield than all cultivars within the dicamba treatment, but 'Florida 91' and 'Red Deuce' plants produced more marketable yield compared with other cultivars treated with 2,4-D. In contrast, 'Mountain Merit' plants produced relatively low marketable yield within each treatment.

The main effects of cultivar and treatment, as well as the interaction of cultivar by treatment, were nonsignificant for the percent nonmarketable yield (p = 0.8978, 0.6403, 0.7803, respectively). For all treatments, the percent nonmarketable yield ranged from 14 to 30%.

Herbicide residue was not detected in any of the fruit samples collected during the third week of harvest.

Table 4. Total fruit yield and marketable yield of tomato cultivars grown treated with an application dicamba or 2,4-D and grown in the field [1].

Cultivar	Total Yield/Plant (g)			Marketable Yield/Plant (g)		
	Control	Dicamba	2,4-D	Control	Dicamba	2,4-D
BHN 589	7950 Ea	7309 Db	7106 Fc	6804 Da	5247 Cb	5570 CDb
Celebrity	9000 Ba	8174 Bb	8169 Bb	7762 Ba	5972 Bb	5952 Bb
Florida 91	9835 Aa	9390 Ab	8339 Ac	8249 Aa	7546 Ab	6613 Ac
Mountain Merit	6093 Fb	6699 Ea	6093 Gb	4411 Ea	4480 Ea	4367 Ea
Primo Red	8738 Ca	6381 Gc	7758 Db	6814 Da	4484 Ec	5618 BCDb
Red Deuce	8699 Ca	7551 Cc	8070 BCb	7301 Ca	5894 Bc	6381 Ab
Red Morning	8555 Da	7349 Dc	7513 Eb	6966 CDa	4851 Dc	5465 Db
Skyway	8778 Ca	6509 Fc	8038 Cb	6791 Da	4030 Fc	5886 BCb
Significance			***p*-value**			
Cultivar (C)		<0.0001			<0.0001	
Treatment (T)		<0.0001			<0.0001	
C × T		<0.0001			<0.0001	

[1] Dicamba and 2,4-D were applied at a drift-simulating rate of 2.8 g ae ha^{-1} and 5.3 g ae ha^{-1}, respectively). WAT = weeks after treatment. Values represent the mean of five replications of each treatment. Treatment means within a column followed by the same uppercase letters and cultivar means within a row followed by the same lowercase letters are not significantly different, according to Fisher's protected LSD test ($p \leq 0.05$).

4. Discussion

Results from these experiments demonstrated that tomato cultivars exposed to a very low rate of dicamba or 2,4-D at a vegetative stage of plant development varied significantly in flowering and fruiting responses to each herbicide compared with nontreated controls. Following exposure to simulated auxin herbicide drift, cultivars in each trial also differed in their fruiting responses. In the greenhouse, marketable yields of herbicide-treated plants of every cultivar were always reduced by 10 to 56% when compared with the respective nontreated control (Table 3). However, 'Red Deuce' plants produced the highest marketable yield, while 'BHN 589' plants had the lowest yield when treated with dicamba. In contrast, 'Primo Red' and 'BHN 589' plants had the highest and lowest marketable yields, respectively, when treated with 2,4-D. Dicamba-treated plants of each cultivar always had more live reproductive organs than the nontreated or 2,4-D-treated plants when the study was terminated (Table 2). Because non-distorted fruit with nondetectable herbicide residue was picked after the first few weeks of harvest, it is likely that these live reproductive organs would produce marketable yield beyond the 14-week period of this study, resulting in some of the cultivars, such as 'Skyway', producing considerably higher yields if the growing season had been extended. The reason for the high number of late-season reproductive organs left on dicamba-treated plants may be attributed to the subsequent development of lateral shoots that produced flowers after the primary stem was initially injured by the herbicide application. Despite this potential recovery of yield for dicamba-treated plants, it would negatively impact the cropping time and delay the subsequent production of another high-value crop, with financial repercussions on cash flow.

In the field experiment, marketable yields of herbicide-treated plants of every cultivar, except 'Mountain Merit', were always reduced up to 41% when compared with the respective control. Unlike the greenhouse experiment, dicamba-treated 'Florida 91' plants produced the highest marketable yield, while 2,4-D-treated 'Florida 91' and 'Red Deuce' plants had the most marketable fruit in the field experiment. Differences in cultivar responses in these environments may be attributed to the capacity of these plants to attain their greater yield potential in the field versus the greenhouse. Although the numbers of reproductive organs on plants at the termination of the field study were not recorded due to the large number of plants, there appeared to be fewer reproductive organs remaining on plants in the field than in the greenhouse.

'Mountain Merit' generally performed poorly when plants were treated with an herbicide or left nontreated. This cultivar was developed for the mountains and Piedmont region of North Carolina and is resistant to fusarium wilt, verticillium wilt, tomato spotted wilt virus, late blight, and root-knot nematodes [28]. Despite its resistance to several important tomato diseases, our results suggest that 'Mountain Merit' is maladapted to the relatively warmer climate in central Missouri, and therefore, it would not be considered a consistent high-yielding tomato cultivar for planting in this area.

'Primo Red', 'Red Morning', 'Skyway', and 'Celebrity' fruit from herbicide-treated plants were initially misshapen compared to tomatoes from nontreated control plants (Figure 4). Although fruit elongation and pointedness were common symptoms during the first three harvests, this type of misshapen fruit was no longer produced on plants exposed to simulated herbicide drift by mid-harvest. Similar observations have been noted on other types of tomato cultivars following plant exposure to auxin herbicides [29]. Genes that control elongation and pronounced tips on the blossom end of fruit are known, and plant exposure to dicamba or 2,4-D apparently results in similar but temporary production of misshapen fruit, which significantly impacts their marketability [30].

No herbicide residue was detected in fruit sampled from the field trial during the third week of harvest. Due to the high cost of residue testing, more extensive sampling of fruit at earlier harvest dates was not conducted. However, elongated fruit from herbicide-treated plants were tested for residue. Presumably, tomatoes that meet the current U.S. grades and standards would be marketable. Previous work with dicamba and 2,4-D has shown a rapid decline in herbicide residue in tomato leaves and fruit when applied at drift-simulating rates [31,32]. When dicamba was applied at 2.2 g ha^{-1}, 30 and 10 ug g^{-1} herbicide residue were detected in tomato plants at 7 and 14 days after treatment, respectively [24]. For plants sprayed with 2,4-D at 11.2 g ha^{-1}, 50 and 10 ug g^{-1} herbicide residue were found in tomato plants at 7 and 14 days after treatment, respectively. No residue was detected by 14 days after treatment when either herbicide was applied at 1.1 g ha^{-1}. Currently, the maximum 2,4-D residue limit in the U.S. is 0.05 ug g^{-1}, but a permissible limit for dicamba has not been established for tomatoes [33]. Despite the lack of detection of residues, the presence of visible symptoms of herbicide injury on tomato plants may result in apprehension to market fruit.

5. Conclusions

This study demonstrated that the severity of injury in response to dicamba and 2,4-D drift is cultivar dependent. In the greenhouse trial, 'BHN 589' and 'Skyway' were among the most sensitive cultivars to a drift-simulating rate of both auxin herbicides for marketable yield, whereas 'Red Deuce' produced relatively high marketable fruit yield following exposure to either herbicide. In the field experiment, the marketable yield of 'Skyway' and 'Red Morning' was reduced substantially by dicamba and 2,4-D, respectively., At the same time, both herbicides caused injury to all tomato plants, field-grown 'Florida 91' plants produced relatively high marketable yield among the cultivars tested after exposure to drift-simulating rates of dicamba, and 'Florida 91' and 'Red Deuce' had high yields following exposure to 2,4-D. Because the type of herbicide drift is often unanticipated, 'Florida 91' may be the preferred tomato cultivar for field cultivation among those tested to maximize potential fruit production. In the future, studies evaluating total soluble solids content and lycopene content would enhance the current knowledge of the effect of these herbicides at drift-simulating rates on tomato fruit quality.

Author Contributions: Conceptualization, M.R.W.; methodology, M.R.W. and R.J.S.; formal analysis, M.R.E.; investigation, M.R.W.; resources, M.R.W.; writing—original draft preparation, M.R.W.; writing—review and editing, M.R.W. and R.J.S.; supervision, M.R.W.; project administration, M.R.W.; funding acquisition, M.R.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Missouri Department of Agriculture Specialty Crop Block Grant Program, grant number AM190100G029.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We gratefully acknowledge Elijah Poehlman and Sarah Dixon for their assistance during the experiments.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Agricultural Marketing Resource Center. Tomatoes. 2021. Available online: https://www.agmrc.org/commodities-products/vegetables/tomatoes (accessed on 15 February 2022).
2. Sonoskie, L.; Culpepper, A.S.; Baxton, L.B.; Richburg, J.S. Evaluating the volatility of three formulations of 2,4-D when applied in the field. *Weed Technol.* **2015**, *29*, 177–184. [CrossRef]
3. Sharkey, S.M.; Williams, B.J.; Parker, K.M. Herbicide drift from genetically engineered herbicide-tolerant crops. *Environ. Sci. Technol.* **2021**, *55*, 15559–15568. [CrossRef] [PubMed]
4. Alves, G.S.; Kruger, G.R.; da Cunha, J.P.; de Santana, D.G.; Pinto, L.A.; Guimarães, F.; Zaric, M. Dicamba spray drift as influenced by wind speed and nozzle type. *Weed Technol.* **2017**, *31*, 724–731. [CrossRef]
5. Bish, M.; Farrell, S.; Lerch, R.; Bradley, K. Dicamba losses to air following applications to soybean under stable and nonstable atmospheric conditions. *J. Environ. Qual.* **2019**, *48*, 1675–1682. [CrossRef]
6. Egan, J.F.; Mortensen, D.A. Quantifying vapor drift of dicamba herbicides applied to soybean. *Environ. Toxicol. Chem.* **2012**, *31*, 1023–1031. [CrossRef]
7. Jones, G.; Norsworthy, K.; Barber, T.; Gbur, E.; Krueger, G. Off-target movement of DGA and BAPMA dicamba to sensitive soybean. *Weed Technol.* **2019**, *33*, 51–65. [CrossRef]
8. Mueller, T.; Steckel, L. Dicamba volatility in humidomes as affected by temperature and herbicide treatment. *Weed Technol.* **2019**, *33*, 541–546. [CrossRef]
9. Nordby, A.; Skuterud, R. The effects of boom height, working pressure and wind speed on spray drift. *Weed Res.* **1974**, *14*, 385–395. [CrossRef]
10. Culpepper, A.S.; Sonoskie, L.M.; Shugart, J.; Leifheit, N.; Curry, M.; Gray, T. Effects of low-dose applications of 2,4-D and dicamba on watermelon. *Weed Technol.* **2018**, *32*, 267–272. [CrossRef]
11. Knezevic, S.Z.; Osipitan, O.A.; Scott, J.E. Sensitivity of grape and tomato to micro-rates of dicamba-based herbicides. *J. Hort.* **2018**, *5*, 229. [CrossRef]
12. Kruger, G.R.; Johnson, W.G.; Doohan, D.J.; Weller, S.C. Dose response of glyphosate and dicamba on tomato (*Lycopersicon esculentum*) injury. *Weed Technol.* **2020**, *26*, 256–260. [CrossRef]
13. Mohseni-Moghadam, M.; Doohan, D. Response of bell pepper and broccoli to simulated drift rates of 2,4-D and dicamba. *Weed Technol.* **2015**, *29*, 226–232. [CrossRef]
14. Bradley, K. Off-Target Movement of Dicamba in Missouri. Univ. Missouri Integrated Pest Management. 2021. Available online: https://ipm.missouri.edu/IPCM/2017/8/Off-target_movement/ (accessed on 22 February 2022).
15. Crowley, B. Hundreds Wait to Have Dicamba Complaints Resolved. News Tribune. 2 March 2022. Available online: https://www.newstribune.com/news/2020/mar/02/Hundreds-wait-to-have-dicamba-complaints-resolved/ (accessed on 22 February 2022).
16. Baurle, M.; Griffin, J.; Alford, J.; Curry, A., III; Kenty, M. Field evaluation of auxin herbicide volatility using cotton and tomato as bioassay crops. *Weed Technol.* **2017**, *29*, 185–197. [CrossRef]
17. Bennet, R.J. The effects of 2,4-D iso-octyl ester/ioxynil herbicide in the liquid and vapour phases on the growth of tomato (*Lycopersicum esculentum* Mill.) plants. *S. Afr. J. Plant Soil* **1989**, *6*, 24–31. [CrossRef]
18. Hemphill, D.D., Jr.; Montgomery, M.L. Response of vegetable crops to sublethal application of 2,4-D. *Weed Sci.* **1981**, *29*, 632–635. [CrossRef]
19. Robbins, W.A.; Taylor, W.S. Injury to canning tomatoes caused by 2,4-D. *Proc. Am. Soc. Hort. Sci.* **1957**, *70*, 373–387.
20. Hermes, M.E. Effects of Simulated 2,4-D and Dicamba Drift on Field-Grown Tomato Plants. Master's Thesis, Texas A& M University, College Station, TX, USA, 2020.
21. Warmund, M.R.; Trinklein, D.H.; Smeda, R.J. Antitranspirants partially mitigate auxin herbicide injury on tomato plants. *HortScience* **2021**, *56*, 932–939. [CrossRef]
22. Coyne, D.P.; Burnside, O.C. Differential plant injury and yield responses of tomato varieties to 2,4-D. *Univ. Neb. Agric. Expt. Stat. Res. Bul.* **1968**, *92*, 226.
23. Zangoueinejad, R.; Alebrahim, M.T.; Tseng, T.M. Evaluation of auxin tolerance in selected tomato germplasm under greenhouse and field conditions. *Weed Technol.* **2019**, *33*, 815–822. [CrossRef]
24. Zangoueinejad, R.; Alebrahim, M.T.; Castro, E.B.; Stallworth, S.; Yue, Z.; Snyder, R.G.; Tseng, T.M. Dose-response study to evaluate dicamba tolerance in selected wild tomato germplasm. *Preprints* **2021**, 2021120011. [CrossRef]
25. United States Department of Agriculture. *United States Standards for Grades of Fresh Tomatoes*; Agriculture Marketing Service. 7 CFR 51; United States Department of Agriculture: Washington, DC, USA, 1991.

26. Trinklein, D. *Fresh Market Tomatoes*; University of Missouri Extension: Columbia, MO, USA, 2016. Available online: https://extension.missouri.edu/publications/g6370 (accessed on 2 February 2022).
27. Wen, C. The Simultaneous Determination of Thirteen Herbicides in Plants by Gas Chromatography/Mass Spectrometry. Master's Thesis, South Dakota State University, Brookings, SD, USA, 1994.
28. Panthee, D.R.; Gardner, R.G. 'Mountain Merit': A late blight-resistant large-fruited tomato hybrid. *HortScience* **2010**, *45*, 1547–1548. [CrossRef]
29. Varten, J.H.M.; Scott, J.W.; Gardner, R.G. Characterization of blossom-end morphology genes in tomato and their usefulness in breeding for smooth blossom-end scars. *J. Am. Soc. Hort. Sci.* **1994**, *119*, 798–803. [CrossRef]
30. Gardner, R.G.; North Carolina State University, Raleigh, NC, USA. Personal communication, 2022.
31. Jordan, T.N.; Romanowski, R.R. Comparison of dicamba and 2,4-D injury to field-grown tomatoes. *HortScience* **1974**, *9*, 74. [CrossRef]
32. Sirons, G.S.; Anderson, G.W.; Frank, R.; Ripley, B.D. Persistence of hormone-type herbicide residue in tissue of susceptible crop plants. *Weed Sci.* **1982**, *30*, 572–578. [CrossRef]
33. Code of Federal Regulations, Part 180 Tolerances and Exemptions for Pesticide Chemical Residues in Food. Available online: https://www.govinfo.gov/content/pkg/CFR-2014-title40-vol24/xml/CFR-2014-title40-vol24-part180.xml#seqnum180.142 (accessed on 23 February 2022).