

## Multi-Year Impact of Dicamba and Glyphosate Herbicides on 'Granny Smith' Apple Trees

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### Abstract

Off-target movement of low-volatile dicamba products has resulted in injury to sensitive plants. An experiment was conducted to determine the effect of dicamba alone or dicamba + glyphosate on tree growth and fruiting of 'Granny Smith'/'Budagovsky 9' (B.9) apple (*Malus domestica* Borkh.) trees over a four-year period. On 8 June 2017, three rates corresponding to  $\frac{1}{2}$ , 1/20, or 1/200 of the manufacturer's labeled rate of dicamba (0.56 kg ae/ha) or dicamba + glyphosate (0.56 kg ae/ha + 1.10 kg ae/ha) were applied to potted one year-old apple trees. On 1 Nov. 2017, trees were transplanted in the field for further evaluation through 2020. Herbicides caused foliar injury on apple trees by three weeks after treatment. Also, trunk diameter growth of trees was reduced by 22 to 45% by herbicide treatments compared with the nontreated control at five months after herbicide exposure. The following growing season, trees treated with dicamba alone or dicamba + glyphosate had 7 to 36% fewer flower clusters, as well as 29 to 63% less cumulative fruit yield in 2020 than control trees. When herbicide treatments were compared, the dicamba + glyphosate treatment resulted in a higher injury rating, a lower number of flower clusters in 2018, and less fruit yield in 2019 than dicamba alone. While the adverse effect of dicamba was generally less than that of dicamba + glyphosate on apple trees, a single dose of these herbicide treatments at driftable rates caused a successive three-year reduction of reproductive organs compared with nontreated controls.

Weed management in apple orchards includes the use of pre- and post-emergence herbicides, which are usually applied as banded, direct sprays under trees. Some of the commonly-used herbicides include dichlobenil, oxyfluorfen, simazine, norflurazon, oryzalin, paraquat, and 2,4-D. Glufosinate and glyphosate are also used as a post-emergence application to actively growing weeds underneath established apple trees in orchards, avoiding contact with root suckers and recent pruning wounds to prevent apple tree injury.

Although several herbicides are registered for use in orchards, dicamba is not labeled for apple. Moreover, dicamba injury has been reported on young apple trees (Dintelmann et al., 2019). An application of dicamba (280 g ae·ha<sup>-1</sup>) + glyphosate (550 g ae·ha<sup>-1</sup>) on one year-old apple trees caused elongation of apple leaves by 28 days after treatment. Also, dicamba alone at 280 g ae·ha<sup>-1</sup> reduced

apple shoot growth by 37% by 112 days after treatment.

With the increasing adoption of dicamba- and glyphosate-tolerant crops, the potential for off-target movement of these auxin herbicides may also become more prevalent. Although best management practices have been developed such as low-volatility formulations of synthetic auxin herbicides, adjuvant and herbicide premixes, and spray nozzles that limit fine spray droplets, injury from drift and volatilization on sensitive crops has been reported by many (Hatterman-Valenti et al., 2017; Knezevic et al., 2018; Kruger et al., 2012; Miller et al., 2020; Mohseni-Moghadam et al., 2016; Warmund et al., 2021).

While some short-term effects of dicamba and glyphosate on young trees have been reported, there is a paucity of information on the long-term consequences of these herbicides on perennial fruit crops. Thus, the

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objective of this study was to evaluate the effect of simulated dicamba and dicamba + glyphosate drift on growth and fruiting of apple trees.

### Materials and Methods

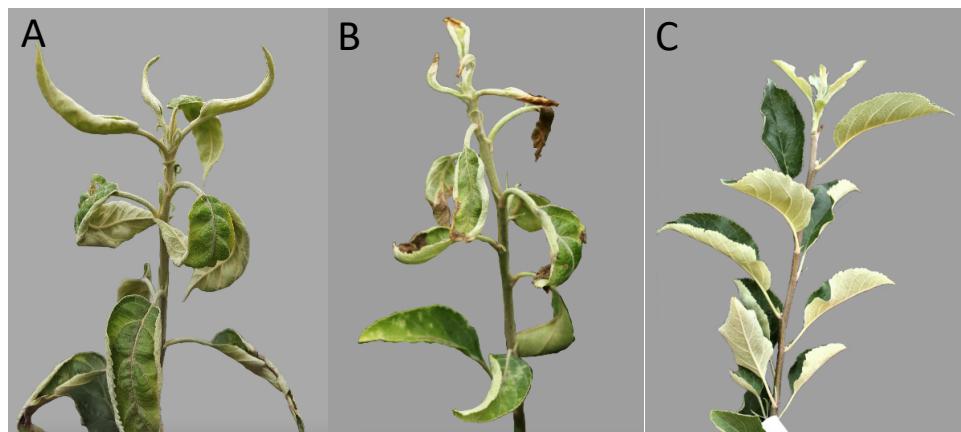
Sixty-five one-year-old dormant 'Granny Smith'/'B.9' trees were obtained from a commercial nursery (Stark Bro's Nurseries & Orchards, Louisiana, MO) on 25 March 2017. Trees were transplanted into 20-L polyethylene containers, using a custom blend potting medium, consisting of 59% pine bark, 9% sphagnum peat moss (BM1; Berger, Saint-Modeste, Quebec, Canada), 6% sand, 6% vermiculite (Therm-O-Rock, Chandler, AZ) and 20% perlite (Therm-O-Rock). Media was supplemented with 1.5 kg controlled-release 38N-0P-0K fertilizer (Nitroform; Koch Turf & Ornamental, Wichita, KS); 1.7 kg micro-nutrient fertilizer (Micromax; Scotts Co., Marysville OH) (6Ca-3Mg-12S-0.10B-1Cu-17Fe-2.5Mn-0.05Mo-1Zn); and 2 kg controlled-release 13N-5.7P-10.8K fertilizer (Osmocote; ICL Specialty Fertilizers, Dublin, OH) per 1.0 m<sup>3</sup> of potting medium on 26 March. Trees were then maintained in an outdoor nursery area at the University of Missouri Horticulture and Agroforestry Research Center (HARC), near New Franklin, MO.

On 8 June 2017, herbicide treatments, including dicamba diglycolamine salt (Xten-dimax with Vapor Grip; Bayer CropScience, St. Louis, MO) alone or dicamba diglycolamine salt + glyphosate (Roundup Powermax, Bayer CropScience) were applied to 'Granny Smith' trees. Three rates corresponding to ½, 1/20, or 1/200 of the manufacturer's labeled rate of dicamba (0.56 kg ae/ha) or dicamba + glyphosate (0.56 kg ae/ha + 1.10 kg ae/ha) for herbicide-tolerant soybean were used. Nontreated control trees were also included for comparison. Herbicides were applied outdoors at 45 cm above the leaf canopy of apple trees using a CO<sub>2</sub>-pressurized backpack sprayer equipped with 8002 XR flat fan nozzles (TeeJet; Spraying Systems, Wheaton, IL) at 140 L·ha<sup>-1</sup> and 131 kPa to simulate drift. After spraying, trees from each treatment were isolated in separate buildings 75 m apart for 72 h without irrigation to minimize vapor movement of herbicides. Following the isolation period, trees were placed back in the outdoor nursery area for the 2017 growing season. The experiment was a randomized complete block design with five, single-plant replications of each treatment. Thereafter, trees received overhead irrigation twice daily during the experiment. Three weeks after herbicide treatment, 20 g of 15N-3.9P-9.9K controlled-release fertilizer (Osmocote; Scotts Company, Marysville, OH) was applied to the medium surface of the potted apple trees.

On 1 Nov. 2017, trees were planted in a deep, upland Menfro silt loam soil (fine-silty, mixed, superactive, mesic typic hapludalfs) at HARC. Trees were spaced 1.8 x 3.7 m apart and were arranged in a randomized complete block design, including 'Gibson Golden Delicious'/'M.9' trees (Stark Bro's Nurseries & Orchards, Louisiana, MO) used for pollination. Thereafter, trees were dormant-pruned and drip-irrigation scheduling and pest and fertility management followed local recommendations (Midwest Fruit Workers, 2017). In 2018 to 2020, weeds underneath apple trees were managed at about a 5 cm height, using a weed-eater weekly during the growing season.

On 1 Apr. 2017, trunk diameter was recorded at 15 cm above the graft union and on 8 Nov. 2017 and 9 Nov. 2020. The increase in trunk diameter was then calculated based on the initial measurement in Apr. 2017. At 3 weeks after treatment (WAT), herbicide injury was estimated, using a rating scale from 0 (no injury) to 10 (tree mortality). The number of flower clusters per tree was recorded in 2018 before their removal by hand. Total fruit weight at harvest was recorded annually and mean fruit weight and cumulative yield for 2019 to 2020 were calculated.

On 25 Oct. 2019, three apples from three replicate trees treated with dicamba at ½ rate



**Fig. 1.** Foliar symptoms of 'Granny Smith' apple shoots three weeks after (A) an application of dicamba alone, (B) dicamba + glyphosate at  $\frac{1}{2}$  the manufacturer's recommended label rate for herbicide-tolerant soybean, or (C) the nontreated control.

were shipped by overnight mail to South Dakota Agricultural Laboratories (Brookings, SD). Dicamba analytes were extracted with dichloromethane for measurement by gas chromatography-mass spectrometry/mass spectrometry, using the method described by Wen (1994) with a quantification limit of 0.5 ppb. On 25 Oct. 2020, three apples from three replicate trees treated with each herbicide treatment at each of the three rates were submitted for dicamba residue analysis as described above.

Data were analyzed using PROC GLIMMIX in SAS (SAS Institute, Cary, NC). Herbicide injury ratings were analyzed as a factorial arrangement of treatments (2 herbicide treatments  $\times$  3 herbicide rates). Although rank transformation was performed on ratings, back-transformed data are presented since results were similar. A Poisson distribution was used to analyze the number of flower clusters. Increase in trunk diameter, fruit weight, average fruit weight, and cumulative yield data were first subjected to a one-way analysis of variance (ANOVA), with all seven treatment combinations, using the PROC GLIMMIX statement in SAS (SAS Institute, Cary, NC). Means were separated by Fisher's protected least significant

difference (LSD) test,  $P < 0.05$ . Next, trunk diameter, flower, and fruit data for nontreated controls were deleted and the remaining data were analyzed as a factorial arrangement of treatments (2 herbicide treatments  $\times$  3 herbicide rates).

## Results

*Vegetative response to herbicides.* Trees treated with either herbicide expressed injury symptoms by 3 WAT. Symptoms were most evident at the  $\frac{1}{2}$  rate of herbicide treatments. Dicamba induced reflexed petioles, as well as curling of entire leaves on the distal portion of shoots (Fig. 1). Injury symptoms caused by dicamba + glyphosate were similar, but foliar necrosis was also visible on trees treated at the  $\frac{1}{2}$  rate.

By 3 WAT, the main effects of treatment and rate were significant for herbicide injury ratings (Table 1). Dicamba + glyphosate treatments had higher ratings compared with dicamba alone. Also, injury ratings were highest when trees were treated at the  $\frac{1}{2}$  rate, intermediate at the  $1/20$  rate, and lowest at the  $1/200$  rate.

Trunk growth of trees was adversely affected by all herbicide treatments (22 to 45% reduction) compared with the nontreated

controls by the end of the first (2017) growing season ( $P = 0.0026$ ). When data were analyzed as a factorial experiment, only the main effect of rate was significant for increase in trunk diameter. Trees treated at a 1/200 or 1/20 rate had a greater increase in trunk diameter than those at the 1/2 rate (Table 1).

Throughout the experiment, tree survival was 100%. Trees treated with dicamba alone or dicamba + glyphosate at the 1/200 rate were the only treatments that had a greater increase (22 or 55%, respectively) in trunk diameter than the controls by Nov. 2020 ( $P < 0.0001$ ). Trees treated with all other herbicide treatment combinations had similar trunk growth as the nontreated controls. Only the main effects of herbicide and rate were significant for increase in trunk diameter when data were analyzed as a factorial experiment (Table 1). Trees treated with dicamba + glyphosate had a greater increase in trunk growth than those treated with dicamba alone by 2020 (Table 1). Also, herbicide-treated trees at the 1/200 rate had a greater increase

in trunk growth than those at the 1/2 rate.

*Reproductive response to herbicides.* In 2018, herbicide treatments reduced the number of flower clusters on tree by 7 to 36% compared with nontreated controls ( $P = 0.0020$ ). The main effects of herbicide and rate were significant for the number of flower clusters when data were analyzed as a factorial experiment (Table 2). Trees treated with dicamba alone produced seven more flower clusters than those treated with dicamba + glyphosate. Trees treated with herbicide at the 1/200 rate had 6 to 9 more flower clusters than those treated with the 1/20 or 1/2 rate, respectively.

For the first cropping year, herbicide treatments applied in 2017 reduced fruit yield per tree by 47 to 69% compared with the non-treated control ( $P < 0.0001$ ). In the factorial analysis, the main effects of herbicide treatment and rate were significant (Table 2). Dicamba + glyphosate-treated trees produced 0.31 kg/tree less fruit weight than those treated with dicamba alone. Also, trees treated at the 1/2 rate had about 0.47 kg less fruit weight

**Table 1.** Herbicide injury ratings and increases in trunk diameter of apple trees treated with herbicides on 8 June 2017.

Treatment	Herbicide injury rating	2017 Increase in trunk diameter (mm)	2020 Increase in trunk diameter (mm)
<b>Herbicide</b>			
Dicamba	1.6 B	5.0 A	24.6 B
Dicamba + glyphosate	1.9 A	4.9 A	29.5 A
<b>Rate</b>			
1/200	0.7 C	5.6 A	31.6 A
1/20	1.4 B	5.3 A	25.6 AB
1/2	3.2 A	4.0 B	24.0 B
Control	---	7.2	22.8
<b>Significance</b>			
Herbicide (H)	0.0104	0.8156	0.0018
Rate (R)	< 0.0001	0.0224	0.0004
H x R	0.5799	0.7143	0.3890

<sup>a</sup>Dicamba and glyphosate were applied at 1/200, 1/20, and 1/2 of the manufacturer's labeled rate (dicamba, 0.56 kg ae·ha<sup>-1</sup> or glyphosate, 1.10 kg ae·ha<sup>-1</sup>) for herbicide-tolerant soybean. Herbicide rating from 0 (no plant injury) to 10 (100% injury) recorded at four weeks after herbicide treatment. Values represent the mean of 5 replications of each treatment. For each of the main effects, means within a column followed by the same letter are not significantly different,  $P \leq 0.05$ . Mean differences are based on a one degree of freedom F-test.

**Table 2.** Number of flower clusters in 2018, annual yield per tree, cumulative yield per tree for 2019 to 2020, and average fruit weight in 2019 of trees treated with herbicides on 8 June 2017.

Treatment	2018 No. flower clusters/tree	2019 Fruit yield/tree (kg)	2020 Fruit yield/tree (kg)	Cumulative yield/tree 2019-2020 (kg)	2019 Average fruit wt. (g)
<b>Herbicide</b>					
Dicamba	42 A	1.88 A	3.20 A	5.07 A	262 A
Dicamba + glyphosate	35 B	1.57 B	3.07 A	4.65 A	267 A
<b>Rate</b>					
1/200	44 A	1.93 A	3.84 A	5.76 A	277 A
1/20	38 B	1.79 AB	3.41 B	5.22 A	264 A
1/2	35 B	1.46 B	2.16 C	3.61 B	253 A
Control	48	3.84	4.45	8.29	291
<b>Significance</b>					
Herbicide (H)	0.0049	0.0488	0.3860	0.0958	0.5489
Rate (R)	0.0135	0.0498	< 0.0001	< 0.0001	0.0646
H x R	0.1842	0.5716	0.2059	0.2062	0.5769

<sup>a</sup>Dicamba and glyphosate were applied at 1/200, 1/20, and 1/2 of the manufacturer's labeled rate (dicamba, 0.56 kg ae·ha<sup>-1</sup> or glyphosate, 1.10 kg ae·ha<sup>-1</sup>) for herbicide-tolerant soybean. Values represent the mean of 5 replications of each treatment. Means within a column followed by the same letter are not significantly different,  $P \leq 0.05$ . Mean differences are based on a one degree of freedom F-test.

than those treated at the 1/200 rate.

In 2020, trees previously treated with any herbicide still had an adverse effect on fruit production, with a 11 to 57% loss of fruit weight compared with the nontreated control. In the factorial analysis, only the main effect of rate was significant for fruit yield (Table 2). Fruit yield per tree was greatest for those treated at the 1/200 rate, intermediate for the 1/20 rate, and lowest for trees receiving the 1/2 rate.

Herbicide treatments reduced cumulative yield by 29 to 63% compared with the nontreated control ( $P < 0.0001$ ). In the factorial analysis, only the main effect of herbicide rate was significant (Table 2). Trees treated with either herbicide at the 1/200 or 1/20 rate produced about 2.1 to 1.6 kg more cumulative yield, respectively, than those at the 1/2 rate (Table 2).

Average fruit weight was adversely affected by herbicide treatments at the 1/20 and 1/2 rates (10 to 14%) compared with the nontreated control in 2019 ( $P = 0.0402$ ). However, average fruit weight from trees treated with dicamba alone (269 g) or dicamba + glyphosate at the 1/200 (285 g) rate

was similar to that for control trees (291 g) in 2019. When average fruit weight data were analyzed as a factorial arrangement of treatments, none of the main effects or the interaction were significant (Table 2). However, in 2020, all herbicides reduced mean fruit size by 5 to 12% compared with nontreated control ( $P < 0.0001$ ). In the factorial analysis, the interaction was significant for average fruit weight in 2020 (Table 3). Mean fruit weight was greater from dicamba + glyphosate-treated trees than from dicamba alone when corresponding rates were compared, except from trees receiving the 1/200 rate of herbicides. Also, for dicamba alone treatments, average fruit weight was greater from trees treated at the 1/200 rate than that from trees treated with the other two rates in 2020. However, for dicamba + glyphosate treatments, average fruit weight from trees treated with the 1/200 or the 1/20 rate was greater than fruit from trees at the 1/2 rate. In 2019, all apple samples from dicamba-treated trees at the 1/2 rate had < 1 ppb residue. The following year, dicamba residue was not detected in any of the apple samples from any herbicide-treated tree.

**Table 3.** Average fruit weight in 2020 on trees treated with herbicides on 8 June 2017.<sup>z</sup>

Herbicide treatment	Average fruit weight (g)		
	1/200	1/20	1/2
Dicamba	270 Aa	253 Bb	250 Bb
Dicamba + glyphosate	265 Ba	262 Aa	258 Ab
Control	285		
Significance		P values	
Herbicide (H)		0.0002	
Rate (R)		<0.0001	
H x R		<0.0001	

<sup>z</sup>Herbicides were applied at 1/200, 1/20, and 1/2 of the manufacturer's labeled rate (dicamba, 0.56 kg ae·ha<sup>-1</sup> or glyphosate, 1.10 kg ae·ha<sup>-1</sup>) for herbicide-tolerant soybean. Means within a column followed by the same uppercase letters and means within a row followed by the same lowercase letters are not significantly different,  $P \leq 0.05$ . Mean differences are based on a one degree of freedom F-test.

## Discussion

Results from this study demonstrated that young apple trees are sensitive to low-dose applications of dicamba alone and in combination with glyphosate following herbicide exposure. Although tree survival was 100% during this study, dicamba herbicide treatments caused foliar injury by 3 WAT (Table 1). Dicamba symptoms included reflexed petioles and curling of leaves (Fig. 1). Although symptoms of dicamba + glyphosate were similar, foliar necrosis was also observed, likely due to the addition of the latter herbicide. However, in the following growing season, foliar injury was no longer apparent. In an earlier study, apple trees treated with dicamba (280 g ae·ha<sup>-1</sup>) + glyphosate (550 g ae·ha<sup>-1</sup>) expressed an estimated 36% injury, which resulted in an increased length:width ratio of apple leaves (5.3) compared with those of nontreated controls (3.3) by 28 DAT (Dintelmann et al., 2019). In the present study, dicamba alone and dicamba + glyphosate also reduced trunk diameters when measured five months after treatment (Table 1). However, by 2020, herbicide-treated trees had similar or greater trunk growth compared with the nontreated controls.

Consequences of herbicide treatments on

reproductive growth were evident for multiple growing seasons (Table 2). In the year after herbicide application, the number of flower clusters was reduced on trees treated with dicamba alone or dicamba + glyphosate compared with the nontreated control. Annual fruit yield losses were also recorded in the following two years, indicating the long-term effect of a single dose of these herbicides beyond the year of treatment on young apple trees.

When herbicide treatments were compared, dicamba + glyphosate was more injurious to apple trees than dicamba alone in terms of higher injury ratings, the lower number of flower clusters, and reduced fruit yield in 2019 (Tables 1 and 2). In 2020, the greater increase in trunk diameter of trees treated with dicamba + glyphosate than dicamba alone also may be associated with low numbers of flowers in 2018 and reduced cumulative fruit yield in 2020 (i.e., low crop load), resulting in greater vegetative growth at the expense of reproductive growth. When average fruit weight from trees treated with dicamba + glyphosate was greater or similar than that for dicamba alone, the number of fruit per tree was also comparatively lower for the dicamba + glyphosate treatment (data

not shown).

Glyphosate injury on apple trees, including directed sprays to root suckers, has been well documented. When glyphosate alone was applied at the manufacturer's recommended rates for apple trees, leaf attenuation, cupping, and chlorosis, as well as stunted shoot growth was reported (Davidson, 1975; Lord et al., 1975; Putnam, 1976). However, leaf removal on non-bearing apple trees within 6 h of glyphosate treatment at the recommended rate prevented injury, whereas defoliation after 12 h from treatment resulted in tree injury (Rom et al., 1977). In Brazil, a reduction in trunk growth and flowering occurred when glyphosate alone was applied to 'Gala' apple trees at the manufacturer's labeled rate in September (Carvalho et al., 2016). When 'MacSpur'/'MM106' apple trees were treated with glyphosate at 6.6 g·L<sup>-1</sup> after harvest in Michigan, flowering was adversely affected during the two subsequent growing seasons (Putnam, 1976). Application of <sup>14</sup>C-glyphosate sprayed directly on the apple surface caused necrotic lesions at the site of application, but the herbicide did not translocate to other plant organs (Putnam, 1976). Although anecdotal reports suggest that glyphosate applications predispose apple tree trunks to *Botrysphaeria dothidea* infection, resulting in canker development, this has not been confirmed in replicated research trials (Rosenberger et al., 2013).

Dicamba residue was detected at a low level (< 1 ppb) in apples sampled from trees treated at the 1/2 rate two years after the herbicide application. However, dicamba at 280 g ae·ha<sup>-1</sup> is considered a relatively high dosage for drift, with a 1/200 rate considered a more common dosage (Kruger et al., 2012). Because we were unable to conduct more extensive testing in the first year of cropping, residue content, if any, in apples from trees sprayed with lower dosages are unknown. By the third growing season following herbicide treatment, dicamba residue was not detected in apples. Currently, a maximum legal dicamba residue limit (i.e., tolerance)

has not been established for apples. However, dicamba residue tolerance for a few other crops, such as asparagus and soybean are 4,000 and 10,000 ppb, respectively (Code of Federal Regulations, 2021). While we did not test for glyphosate, the legal tolerance for glyphosate in pome fruits is 200 ppb (Code of Federal Regulations, 2021).

In conclusion, young 'Granny Smith' apple trees are sensitive to low dose applications of dicamba and dicamba + glyphosate, which can cause reproductive losses in successive growing seasons. In Missouri, young apple trees are actively growing with rapid foliar expansion during late-May to early June, which coincides with the postemergence applications of dicamba alone or dicamba + glyphosate to transgenic soybeans. Thus, off-target herbicide movement of dicamba alone or with glyphosate from herbicide-tolerant soybean fields may cause apple crop loss on young trees in orchards nearby.

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