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Effect of simulated 2,4-D and dicamba drift on strawberry (*Fragaria* × *ananassa*) plant and fruit development

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Abstract

Greenhouse studies were conducted from 2020 to 2021 to evaluate the effect of simulated drift rates of 2,4-D and dicamba on strawberry growth, fruit development, and fruit quality in Raleigh, NC. Treatments included 2,4-D choline and dicamba DGA plus Vapor Grip at 1/2×, 1/20×, and 1/200× of the 1× field rate of 1.09 and 0.8 kg ae ha^{-1}, respectively. Treatments were applied to strawberry at three reproductive stages, including bud, flower, and fruit. Averaged across both herbicides, strawberry canopy size was reduced by the 1/2× rate 18, 25, 30, and 36% at 3, 6, 9, and 11 wk after treatment (WAT). The 1/2× rate of both herbicides caused greater injury to strawberry than the 1/20× or 1/200×, with maximum stunting from 2,4-D and dicamba of 54% and 36%, respectively. Fruit pH and total soluble solid content (SSC) increased due to the 1/2× rate of dicamba compared to the 1/20× and 1/200× rates and the nontreated. Treated fruit (across all herbicides) were larger than fruit developing following herbicide application to flowers or buds but were similar to nontreated fruit.

Introduction

The use of synthetic auxin herbicides has increased in resistant agronomic crops such as cotton (*Gossypium hirsutum* L.) and soybean [*Glycine max* (L.) Merr.] to control weeds [Palmer amaranth (*Amaranthus palmeri* S. Wats.); ragweed (*Ambrosia artemisiifolia* L.); horseweed [(*Conyza canadensis* (L.) Cronq.)] resistant to acetolactate synthase inhibitors, glyphosate, and protoporphyrinogen oxidase inhibitors (Green 2012; Heap 2021). There are more than 360 unique cases of weeds resistant to these three herbicides in the United States (Heap 2021). The increased use of synthetic auxin herbicides has raised concerns about the potential for off-target movement of these herbicides to sensitive crops. Injury from low doses of synthetic auxin herbicides has been observed in cantaloupe (*Cucumis melo* var. *cantelupo* Ser.) and cucumber (*Cucumis sativus* L.) (Hand et al. 2021), cotton (Marple et al. 2008), grape (*Vitis vinifera* L.) (Mohseni-Moghadam et al. 2016), and tomato (*Solanum lycopersicum* L.) (Fagliari et al. 2005; Kruger et al. 2012).

Low-dose studies seek to evaluate the effect on off-target species from simulated particle and/or vapor drift. Drift is defined as "airborne movement of pesticides from an area of application to any unintended site," which can occur during application as particle drift or after application as vapor drift (NPIC 2017). Simulated drift studies that have been conducted vary the treatments including herbicides, rates, number of applications, and crop stage at application. Many of these studies consider off-target movement of herbicides to vegetable crops like broccoli (*Brassica oleracea* L. var. *italica*) (Mohseni-Moghadam and Doohan 2015), yellow squash (*Cucurbita pepo* L.) (Dittmar et al. 2016), bell pepper (*Capsicum annum* L.) (Dittmar et al. 2016; Mohseni-Moghadam and Doohan 2015), cucumber (Gilreath et al. 2001; Hand et al. 2021), and watermelon [*Citrullus lanatus* (Thunb.) Matsum and Nakai] (Culpepper et al. 2018). Effects of simulated herbicide drift on fruit crops such as grape (Mohseni-Moghadam et al. 2016), cherry (*Prunus avium* L.) (Bhatti et al. 1995), apple (*Malus* × *domestica* Borkh.), and pear (*Pyrus communis* L.) (Carvalho et al. 2016) have also been reported. Data collected were similar across these studies with results explaining the impact of the simulated drift on visible crop injury and yield, but few studies report marketability of the yield and only one has reported fruit quality (Bhatti et al. 1995).

Figure 1. Tagged reproductive stages: (left) buds, (center) flowers, (right) fruit.

Research by Dintelmann et al. (2019) observed the effects of low doses of the synthetic auxins 2,4-D and dicamba on vegetative strawberry. Strawberry was reported to be more sensitive to 1/2× rate of dicamba than 1/2× rate of 2,4-D (1× rate of dicamba is 0.56 kg ae ha^{-1}; 1× rate of 2,4-D is 1.09 kg ae ha^{-1}), causing 24% and 3% injury, respectively, 28 d after treatment (DAT). No injury was observed from any rates of 2,4-D or dicamba 112 DAT. Strawberry crop tolerance to 2,4-D when applied during the vegetative stage has also been reported by McMurray et al. (1996), with 4% injury observed on strawberry plants at the 9- to 10-leaf stage 6 wk after treatment (WAT). Sims et al. (2022) observed no differences in strawberry crop injury when 2,4-D was applied at a maximum rate of 2.13 kg ha^{-1}. No differences were reported in shoot length for any rates compared to the nontreated control (Dintelmann et al. 2019). The Dintelmann et al. (2019) study did not assess the effect of low doses of 2,4-D and dicamba on fruit development.

Strawberry is a high-value crop in the United States with a farm-gate value of over $2.5 billion and over 17,800 ha harvested in 2019, with California and Florida ranked the top one and two producing states, respectively (USDA 2020). 2,4-D choline as Enlist One (Dow AgroScience, Indianapolis, IN) is registered for use in corn (*Zea mays* L.), cotton, and soybean, where it can be applied as a preplant burndown or until V8 stage in corn, full-flowering in cotton, and R2 stage in soybean (Anonymous 2021). The average planting date for these crops ranges from late March to mid-May, which coincides with strawberry reproduction and harvest (C. Cahoon, personal communication; Dunphy 2018; Edmisten and Collins 2021; Poling et al. 2005). As synthetic auxin-resistant agronomic crops continue, production near specialty-crop fields, like strawberry, is inevitable, and the potential for drift could affect growth, fruit yield, and fruit quality. This study aims to evaluate the effect of simulated 2,4-D and dicamba drift at three reproductive stages (bud, flower, and fruit) on strawberry growth, fruit development, and fruit quality.

Materials and Methods

Single-rooted day-neutral strawberry cultivar 'Albion' plug plants were planted on September 30, 2020, as a single-rooted plug plant into 2.8-L black plastic pots filled with potting soil (Jolly Gardener Pro-Line C/P Growing Mix, Oldcastle APG, Inc., Atlanta, GA). Plants were fertilized every other wk with 24-8-16 water-soluble fertilizer (Miracle-Gro All Purpose Plant Food, Scotts Miracle-Gro Products, Marysville, OH) until termination.

Treatments included 1/2×, 1/20×, and 1/200× of the 1× field rate of 1.09 and 0.8 kg ae ha^{-1} for 2,4-D choline (Embed Extra, Corteva Agriscience, Indianapolis, IN) and dicamba DGA plus Vapor Grip (XtendiMax plus Vapor Grip, Monsanto Co., St. Louis, MO), respectively. Treatments were applied at the reproductive stages bud (petals enclosed by sepals), flower (petals fully expanded and still attached), and fruit (full petal fall) (Figure 1). One day before herbicide application, five stems of each reproductive stage per plot were tagged with a zip tie, and a specific color zip tie was used for each reproductive stage. Effort was taken to tag primary and secondary stages within an inflorescence to ensure uniformity of mature fruit size across the experiment, and all tagged stages were used for data collection.

Herbicide treatments were applied over the top of the strawberry plants on December 21, 2020 (run 1) and December 22, 2020 (run 2) using a CO_2-pressurized backpack sprayer calibrated to deliver 140 L ha^{-1} with one TeeJet XR11002 VS flat-fan nozzle (TeeJet Technologies, Springfield, IL). After leaves dried from treatment application, strawberry plants were moved to Marye Anne Fox Teaching Laboratory Greenhouses (35.7871°N, 78.6729°W) at North Carolina State University, Raleigh, NC under natural light conditions and a temperature range between 24 and 27 C for the remainder of the study. Experiments were separated in time by one day to ensure that the reproductive growth of both experiments was consistent.

The experiment was a split-plot factorial plus a nontreated control arranged in a randomized complete block. Treatments were replicated five times, and the experiment was conducted twice (2020–2021). The whole plots were two herbicides and three rates, and the subplots were three strawberry reproductive stages. Each whole plot consisted of three pots, with all three reproductive stages present across each plot. Blocks were arranged north to south to account for a sun exposure gradient.

Data recorded included visible crop injury 2, 7, 14, 21, 28, 42, and 56 (DAT). Crop injury was characterized by plant stunting and deformation, chlorosis and necrosis of the leaves, and rated on a scale of 0 (no injury) to 100% (crop death) (Frans et al. 1986). Strawberry canopy was measured 1 wk before the herbicide application, and then every 3 wk by measuring the height, the widest part of the canopy and the width perpendicular. Measurements were multiplied together to obtain the approximate canopy size.

Tagged strawberry fruit were hand harvested as they ripened (at least 75% red) beginning January 6, 2021 (Gross et al. 2016; USDA 2006). Each fruit was weighed using a Scout SPX421 g scale (Ohaus Corporation, Parsippany, NJ), and fruit dimensions of length, width and thickness were measured using a digital caliper (Digimatic Caliper CD-6" CSX, Mitutoyo Corp., Kawasaki, Japan), and averaged within each plot by reproductive stage. Fruit dimensions [length (L), width (W), and thickness (T)] were used to calculate additional external fruit traits including geometric mean diameter (D_g) [Eq. 1], sphericity (Ø) [Eq.2], surface area (S) [Eq.3], and fruit shape index (SI) [Eq.4] (Morais et al. 2019).

Table 1. Effect of low-dose rates of 2,4-D and dicamba on strawberry canopy size, averaged across herbicides in greenhouse studies held in Raleigh, NC, 2020 to 2021.[a,b]

	Strawberry canopy size			
Rate[c]	3 WAT[d]	6 WAT	9 WAT	11 WAT
	m^3			
0	0.017 b	0.015 b	0.014 b	0.014 b
1/200	0.017 b	0.015 b	0.014 b	0.014 b
1/20	0.017 b	0.014 b	0.015 b	0.014 b
1/2	0.014 a	0.011 a	0.010 a	0.009 a
P value	0.0002	<0.0001	<0.0001	<0.0001

[a]Strawberry canopy was determined by measuring the height, then widest part of the plant canopy and then the width perpendicular.
[b]Means within a column followed by the same letter are not different according to Tukey's HSD (α = 0.05).
[c]1× rate of 2,4-D and dicamba were 1.09 and 0.8 kg ae ha^{-1}, respectively.
[d]Abbreviations: WAT, wk after treatment.

$$D_g = (LWT)^{1/3} \quad [1]$$

$$Ø = D_g/L \quad [2]$$

$$S = \pi(D_g)^2 \quad [3]$$

$$SI = L/W \quad [4]$$

Fruit was assessed for marketability as marketable (fully developed and uniform size) and cull (Gross et al. 2016; USDA 2006). Marketable fruit was stored by plot and reproductive stage treatment in plastic storage bags and stored at −20 C until fruit were analyzed for fruit quality. Frozen strawberry samples were thawed to room temperature, then homogenized by hand crushing, and the juice was filtered through filter paper (Fisherbrand Filter Paper Qualitative P8, Fisher Scientific Company, Pittsburg, PA). Each homogenized sample was analyzed for total soluble solid content (SSC), titratable acidity (TA) [percent citric acid equivalents (v/v)], and pH. SSC and TA were determined by the PAL-BX|ACID F5 pocket Brix-acidity meter (Atago Company Ltd., Bellevue, WA) on setting 4 for strawberry. The pH of each fruit sample was measured using a PC800 pH meter (Apera Instruments, Columbus, OH) standardized to pH 4 and 7. Not all plots had an adequate number of fruit for a given reproductive stage to analyze fruit.

On March 1, 2021, after all tagged fruit were harvested, all remaining reproductive structures (fruit, flowers, and buds) were removed from each plant (subplot) and counted. Counts were averaged across the plot (flower count). Shoot fresh weight (SFW) was taken of each plant per plot by cutting the plant at the base of the crown near the soil and weighed using a Scout SPX421 g scale. Each plant shoot was placed in a brown paper bag and put into a dryer for 72 h at 74 C. Shoots were removed, weighed using a Scout SPX421 g scale and dry weights recorded. SFW and shoot dry weight (SDW) were averaged across the plot.

Data were subjected to ANOVA and analyzed using SAS PROC GLIMMIX (SAS 9.4, SAS Institute, Cary, NC). Fixed effects were herbicide, rate, reproductive stage, experiment, and respective interactions, and random effects included replication within experiment and replication by reproductive stage within herbicide by rate (subplot error). The nontreated control was not included in visible crop injury analysis, but this treatment was included for all other analyses. Shoot fresh and dry weights and flower count data were calculated as percent of the nontreated. Means were separated using Tukey's HSD (α = 0.05).

Results and Discussion

Data were pooled across experiments for analyses of dependent variables as a result of lack of treatment–by–experiment interaction. Data were combined across herbicides for strawberry canopy size, because rate alone was significant. The 1/2× rate combined across herbicides reduced strawberry canopy size 18%, 25%, 30%, and 36% of nontreated at 3, 6, 9, and 11 WAT, respectively (Table 1). Canopy size from 2,4-D and dicamba at 1/20× and 1/200× rates were similar to the nontreated.

A significant rate-by-herbicide interaction was observed for strawberry plant stunting and leaf deformity at all rating dates, except for 21 and 28 DAT leaf deformity ratings when only rate was significant (Table 2). The 1/2× rate of 2,4-D and dicamba was more injurious to strawberry than the 1/20× or 1/200×, with maximum stunting of 54% and 36% from 2,4-D and dicamba, respectively, 56 DAT. 2,4-D at the 1/2× rate caused up to 7% leaf deformity 42 DAT, but injury was transient because by 56 DAT only 1% deformation was observed. At the 1/2× rates, dicamba caused greater leaf deformation than 2,4-D, with a maximum deformation of 26% at 56 DAT (Table 2, Figure 2). These results were similar to those reported by Dintelmann et al. (2019).

Leaf chlorosis was not observed until 28 DAT, at which time a rate-by-herbicide interaction occurred with dicamba; the 1/2× rate of dicamba caused 1% leaf chlorosis, and all other treatments were 0 (P value 0<.0001, data not shown). Leaf necrosis was only significant 14 and 28 DAT, where a rate-by-herbicide interaction occurred, and the 1/2× rate of 2,4-D caused 5% and 3%, respectively ($P < 0.0001$, both; data not shown).

Rate alone was significant for SFW, SDW, and flower count; therefore, data were combined across herbicides (Table 3). SFW and SDW from the 1/2× rate (68% and 66% of the nontreated, respectively) was less than SFW and SDW in the 1/20× or 1/200× treatments. Flower count was lower in the 1/2× rate treatment (72% of the nontreated) than the 1/200× rate treatment (102% of the nontreated), but similar to the 1/20× rate treatment (89% of the nontreated).

Main-effects rate and reproductive stages were significant for fruit size parameters, and data were combined across nonsignificant effects (data not shown). However, whereas some data for fruit size parameters were statistically significant, most differences biologically were not significant. Fruit weight was greater for treated fruit (12 g) than treated buds (8.4 g), but neither were different from treated flowers and all nontreated stages. Fruit weight may have been affected by selection of reproductive stages prior to application, as the fruit stage would have been composed of more primary fruiting structures on the inflorescences and buds could have been more secondary fruiting structures, thus producing smaller fruit at harvest (Poling n.d.). The difference in weight from a commercial harvest standpoint is also negligible.

Fruit size (length, width, and thickness) was minimally significant biologically; treated fruit were comparable to length of fruit developing from nontreated buds, and width and thickness of fruit developing from nontreated buds and flowers (data not shown). Strawberry plants treated with 2,4-D developed longer fruit at harvest regardless of reproductive stage at application than dicamba, but neither were different from the nontreated (data not shown) and the less than 2 mm difference between the two herbicides would not be visibly different. Fruit width and thickness was greater in the 1/20× rate treatment (30 and 26 mm, respectively) compared to the 1/2× rate treatment (27 and 23 mm, respectively), but comparable to the 1/200× rate and nontreated treatments (data not shown). Thus, plants receiving

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Table 2. Percent injury of low-dose rates of 2,4-D and dicamba on strawberry plant stunting and leaf deformation, in greenhouse studies held in Raleigh, NC, 2020 to 2021.[a,b]

Herbicide	Rate[b]	Stunting[c]							Leaf deformity				
		2	7	14	21	28	42	56	14	21[e]	28	42	56
		DAT[d]											
		%											
2,4-D	1/200	2 a	1 a	0 a	1 a	2 a	2 a	3 a	0 a	0 a	0 a	0 a	0 a
	1/20	3 a	1 a	0 a	2 a	3 a	10 b	7 a	0 a	0 a	0 a	0 a	0 a
	1/2	16 b	22 b	23 b	23 b	36 b	48 c	54 b	6 b	4 b	7 b	7 b	1 a
P value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0011	0.6084
Dicamba	1/200	2 a	1 a	4 ab	1 a	2 a	6 a	5 a	0 a			0 a	0 a
	1/20	3 a	1 a	0 a	2 a	4 a	6 a	7 a	0 a			1 a	0 a
	1/2	3 a	8 b	0 a	8 b	14 b	31 b	36 b	2 b			18 b	26 b
P value		0.5154	<0.0001	0.0626	0.0009	<0.0001	<0.0001	<0.0001	<0.0001			<0.0001	<0.0001

[a]Means within columns by herbicides with the same letter are not statistically different according to Tukey's HSD (α = 0.05). The nontreated was not included in the statistical analysis.
[b]1× rate of 2,4-D and dicamba were 1.09 and 0.8 kg ae ha^{-1}, respectively.
[c]Strawberry plant stunting and leaf deformation were recorded on a scale of 0 (no injury) to 100 (plant death).
[d]Abbreviations: DAT, d after treatment.
[e]Rate alone was significant for leaf deformity at 21 and 28 DAT; injury averaged across herbicides.

Figure 2. Foliar injury to strawberry plant from 1/2x rate of dicamba treatment 56 d after treatment.

Table 3. Effect of low-dose rates of 2,4-D and dicamba on strawberry shoot fresh and dry weight, and flower count, combined across herbicides, in greenhouse studies held in Raleigh, NC, 2020 to 2021.[a,b]

Rate[c]	Shoot fresh weight	Shoot dry weight	Flower count
	% of nontreated		
1/200	97 b	97 b	102 b
1/20	97 b	96 b	89 ab
1/2	68 a	66 a	72 a
P value	<.0001	<.0001	.0049

[a]Means within a column followed by the same letter are not different according to Tukey's HSD (α=0.05).
[b]Shoot fresh weight, shoot dry weight, and flower count from nontreated treatment was 45.96 g, 11.04 g, and 23.8, respectively.
[c]1x rate of 2,4-D and dicamba were 1.09 and 0.8 kg ae ha^{-1}, respectively.

the 1/2× rate treatment of 2,4-D or dicamba may result in narrower fruit (Figure 3).

Geometric mean diameter (GMD) and surface area (SA) were larger in treated fruit (28 mm and 26 cm^2, respectively) than in treated flowers (24 mm and 21 cm^2, respectively) and buds (24 mm and 20 cm^2, respectively), but similar to GMD of nontreated fruit and buds, and SA of all nontreated stages (data not shown). As with weight, these differences could result from inflorescence structure selection before application and would be insignificant to the commercial industry. Although there were statistical differences in values for sphericity (rate and reproductive stage) and shape index (SI) (rate), they are not substantial enough to

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Figure 3. Elongation of strawberry fruit, developed post-application, from 1/2x rate of dicamba treatment.

Table 4. Effects of low-dose rates of synthetic auxins and reproductive stages of strawberry on pH, titratable acidity (TA), and soluble solid content (SSC) of strawberry fruit.[a]

Dependent variable		pH	TA[c]	SSC[d]
Herbicide	Rate[b]			
Nontreated		3.37 a	1.28 a	8.5 a
2,4-D	1/200	3.41 a	1.30 a	9.2 a
	1/20	3.38 a	1.31 a	9.0 a
	1/2	3.40 a	1.26 a	8.9 a
Dicamba	1/200	3.39 a	1.28 a	8.6 a
	1/20	3.40 a	1.26 a	9.3 a
	1/2	3.48 b	1.23 a	10.6 b
p-value		.0036	.8593	<.0001
Reproductive stage				
Fruit	Treated	3.47 b	1.37 b	9.3 a
Flower	Treated	3.39 a	1.26 a	9.4 a
Bud	Treated	3.37 a	1.21 a	9.2 a
Fruit	Nontreated	3.42 ab	1.34 ab	8.8 a
Flower	Nontreated	3.34 a	1.22 a	8.2 a
Bud	Nontreated	3.34 a	1.30 a	8.6 a
p-value		<.0001	<.0001	.7667

[a]Means within columns by dependent variable (herbicide by rate or reproductive stage) with the same letter are not statistically different according to Tukey's HSD (α=0.05).
[b]1x rate of 2,4-D and dicamba were 1.09 and 0.8 kg ae ha^{-1}, respectively.
[c]TA is measured in percent citric acid equivalents (v/v).
[d]SSC is expressed in °Brix.

suggest low doses of 2,4-D or dicamba affect sphericity or SI of strawberry fruit (data not shown).

A rate-by-herbicide interaction was observed for strawberry fruit pH and SSC (Table 4). Fruit from the 1/2× rate of dicamba treatment had a higher pH (3.48) and SSC (10.6) than the other treatments including the nontreated, suggesting a misapplication of dicamba could alter the pH and SSC of strawberry fruit. However, these increases are minimal in respect to production standards (P. Perkins-Veazie, personal communication). Specific to the reproductive stage, treated fruit had higher pH (3.48) and TA (1.37) than fruit developing from treated flowers and buds, and nontreated flowers had higher pH than nontreated buds (Table 4). Although statistically significant, the fruit in the nontreated treatment was similar to all other treatments. This observation may suggest that before herbicide application the fruit quality composition of the fruit stage was different than the flower or bud stages. Due to the inadequate number of fruit in some plots for analyses, these data would likely be better represented if collected from a field study.

Findings from this study on strawberry visible injury were similar to those of Dintelmann et al. (2019), but our study also evaluated the effects of low-dose rates of 2,4-D and dicamba as they relate to reproductive development. Flower count decreased relative to the nontreated, with a 1/2× rate treatment of 2,4-D and dicamba, and this was probably due to the smaller-sized plants at the time of study termination. Fruit quality parameters may be affected by low doses of 2,4-D and dicamba, but low sample sizes for fruit analysis prohibit confirmation. Fruit size parameters (weight, width, thickness, GMD, and SA) did indicate a larger fruit was produced by plants receiving the 1/20× rate compared to plants receiving the 1/2× rate of either 2,4-D or dicamba, but specific to the reproductive stages, treated stages were not different from their nontreated counterparts.

Practical Implications

Findings from this study indicate that low-dose rates of 2,4-D and dicamba can affect strawberry plant stunting and leaf formation, fruit size, and fruit quality. With the potential for 2,4-D as Embed Extra to be registered for use in strawberry, future research should be conducted in the field to provide a larger fruit sample size for yield (especially marketable yield), fruit quality analysis (pH, TA, SSC), and residue sampling. An additional study could determine if auxin herbicide drift affects ripening, as studies suggest auxin hormones play a role in fruit ripening (Given et al. 1988; Liu et al. 2011).

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