Journal of the American Pomological Society 75(4): 214-227 2021

Auxin Herbicide Drift Injury on American Elderberry Plants Influenced by Growth Stage

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Additional index words: dicamba, drift injury, fruit quality, 2,4-D, postemergence herbicides, *Sambucus nigra* subsp. *canadensis*.

Abstract

The use of low-volatile 2,4-D and dicamba products on herbicide-tolerant crops has resulted in cases of off-target movement and injury to sensitive plants. An outdoor nursery and a greenhouse experiment were conducted to determine the herbicide sensitivity of potted American elderberry [*Sambucus nigra* L. subsp. *canadensis* (L.) Bolli] plants at a vegetative (V), tight green cluster (TGC), or full bloom (FB) stage. For the nursery experiment, one rate corresponding to 1/20 of the manufacturer's labeled rate of 2,4-D (1.06 kg ae·ha^{-1}) or dicamba (0.56 kg ae·ha^{-1}) was applied, whereas 1/20 and 1/200 rates of these herbicides were used in the greenhouse study. For plants treated at the V stage, necrosis was observed at the stem apex of some canes treated with either herbicide at the 1/20 rate. For the 1/200 rate, 2,4-D applied at the V stage resulted in a slight hormetic effect on cane growth compared with the nontreated control by 8 weeks after treatment (WAT). Elderberry plants were generally more sensitive to floral abortion when treated with an auxin herbicide at TGC versus the FB stage. For the 1/20 rate in both studies, 2,4-D caused floral distortion or complete abortion of umbels of 50 to 100% of canes, whereas dicamba caused complete abortion on 10 to 40% of the canes when applied at the TGC stage. For the remaining canes that produced fruit, yield and juice/umbel was reduced by both herbicides at the 1/20 rate. In contrast, herbicides applied at the 1/200 rate at TGC had no effect on fruit yield and juice/umbel compared with the nontreated control in the greenhouse study. Also, plants treated at TGC with 2,4-D at the 1/200 rate (no fruit produced on plants at the 1/20 rate) or dicamba at both rates had lower juice soluble solids concentration (SSC) and SSC/titratable acidity ratios than those from nontreated controls. Herbicide residue was present in juice from all fruiting plants when treated at TGC or FB, but the amount detected in juice from plants exposed at FB to 2,4-D at the 1/20 rate (391 ppb) exceeded the allowable tolerance level for elderberry.

Elderberry is a niche crop in North America with flowers used as a flavorant in beverages, whereas juice extracted from fruit is primarily used for culinary or medicinal purposes. American elderberry [*Sambucus nigra* L. ssp. *canadensis* (L.) Bolli] plants are multi-stemmed shrubs with flowers borne on current season's growth in compound umbels in early summer (Charlebois et al., 2010; Warmund et al., 2019; Zomlefer, 1994). Fruit is harvested in late July and August. For commercial plantings, typical fruit yields from improved elderberry cultivars are 9,000 to 14,000 kg·ha^{-1} (Finn et al., 2008). Although plant productivity can be affected by pruning frequency, yield potential may be limited by low fruit set, uneven ripening within the umbel, pests, and other abiotic factors (Thomas et al., 2009).

One of the challenges to elderberry production, as well as other fruits and vegetables, has been plant injury due to particle drift or volatility of auxin herbicides (Hatterman-Valenti et al., 2017; Hemphill and Montgomery, 1981; Knezevic et al., 2018; Kruger et al., 2012; Miller et al., 2020; Mohseni-Moghadam and Doohan, 2015; Mohseni-Moghadam et al., 2016). With the recent introduc-

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Auxin Herbicide Drift Injury on Elderberry Plants Influenced by Growth Stage tion of 2,4-D and dicamba tolerant crops, the frequency of damage to sensitive crop plants has increased. Although stewardship technologies have been developed, including low-volatility formulations of synthetic auxin herbicides, adjuvant and herbicide premixes, and spray nozzles that limit fine spray droplets, injury from drift and volatilization has continued on sensitive crops. Off-target movement of spray material is affected by ambient temperature, relative humidity, vapor pressure, and wind speed (Alves et al. 2017; Bish et al., 2019; Egan and Mortensen 2012; Jones et al., 2019; Mueller and Steckel 2019; Nordby and Skuterud 1974).

Herbicide injury on elderberry canes was reported when plants were treated before flowers were visible with 2,4-D or dicamba at 545 or 280 g ae·ha^{-1}, respectively (Dintelmann et al., 2019). Injury symptoms were also noted on a few canes that later flowered and produced fruit during this study (M.R. Warmund, unpublished data). Other studies on annual crops, including tomato and herbicide-sensitive soybeans, demonstrated greater yield losses when low rates of auxin herbicides were applied at early bloom versus post-bloom stages (Fagliari et al., 2005; Griffin et al., 2013; Kruger et al., 2012; McCown et al., 2018; Scholtes, 2014).

While studies have been conducted on the effect of synthetic auxin herbicide injury on annual herbaceous crops, there is a paucity of information for perennial fruit crops. Thus, the objective of this study was to evaluate the effect of simulated 2,4-D and dicamba drift injury on the cane growth, fruiting, and juice characteristics of American elderberry plants treated at three stages of growth.

Materials and Methods

Outdoor nursery experiment. Two-year-old American elderberry plants were obtained from a commercial nursery (Forrest Keeling Nursery, Elsberry, MO) on 4 May 2018. Plants were transplanted into 10-L polyethylene containers, using ProMix BX (Premier Tech Horticulture, Quakertown, PA). Canes were then pruned to 30 cm each above the medium surface with four canes per container. Three weeks before treatment, 50 g of 15N-3.9P-9.9K controlled-release fertilizer (Osmocote; Scotts Company, Marysville, OH) was applied to the medium surface of the potted elderberry plants. On 1 June 2018, herbicide treatments were applied to non-flowering vegetative (V) plants and those at the tight green cluster (TGC) stage. Herbicide treatments included dicamba diglycolamine salt (Xtendimax with Vapor Grip; Bayer, St. Louis, MO) or 2,4-D choline (Enlist One with Colex-D; Dow AgroSciences, Indianapolis, IN) at 1/20 of the manufacturer's recommended use rate (0.56 kg ae·ha^{-1}) or 2,4-D (1.06 kg ae·ha^{-1}). Similar treatments were applied to plants at full bloom (FB) on 21 June 2018. Nontreated control plants were also included for comparison. Herbicides were applied outdoors at 43 cm above the leaf canopy of elderberry plants using a CO_2-pressurized backpack sprayer equipped with 8004 flat fan nozzles (TeeJet Technologies, Urbandale, IA) at 140 L·ha $^{-1}$ and 193 kPa to simulate drift. After spraying, plants from each treatment were isolated in separate buildings 75 m apart for 72 h without irrigation to minimize vapor movement of herbicides. Plants were then arranged in a randomized complete block design with five, single-plant replications of each treatment in an outdoor nursery area at the Horticulture and Agroforestry Research Center, near New Franklin, MO. Thereafter, plants received overhead irrigation twice daily during the experiment.

Cane length was recorded at treatment and 8 WAT for plants treated at V and TGC stages and the increase in length was calculated. Because very little or no cane elongation occurred after the FB stage, cane lengths were not recorded for plants at the FB stage. Fruit was harvested at peak ripeness (i.e., all fruit in umbel a dark purple color). Fruit weight/cane and drupe number/cane were recorded and mean drupe weight was calculated. Immediately after harvest, berries were washed, destemmed, and pressed in a sieve for juice extraction. Juice per umbel was recorded and juice per drupe was determined before storing samples at -22 °C. For plants treated at TGC, soluble solid concentration (SSC), pH, and titratable acidity (TA) were not evaluated due to the limited amount of juice from herbicide-treated plants. To evaluate juice characteristics of plants treated at FB, samples were thawed and pooled by plant. Because some treatments produced a small quantity of juice, each sample was diluted with a similar volume of double-distilled water before further analyses. A 0.3-mL aliquot of juice was used to determine SSC with a digital refractometer (Atago USA, Bellevue, WA) and a 10 mL aliquot was used to measure pH (HI222; Hanna Instruments Woonsocket, RI). To measure TA, a 10 mL aliquot was diluted with 48 mL of degassed deionized water and titrated (G20 Compact Titrator; Mettler-Toledo, Columbus, OH) to 8.2 pH with 0.1N sodium hydroxide. Titratable acidity, expressed as citric acid, was then calculated. The SSC/TA ratio was calculated to determine the balance of sugars and acid.

Cane length data from plants treated at the non-flowering and the TGC stage were analyzed as a factorial experiment (3 herbicide treatments x 2 stages). All other data from the TGC stage were subjected to a one-way analysis of variance (ANOVA) using the PROC GLIMMIX statement in SAS (SAS Institute, Cary, NC). Means were separated by Fisher's protected least significant difference (LSD) test, $P < 0.05$. Data collected from plants treated at the FB stage were analyzed separately as a one-way ANOVA with mean separation by Fisher's protected LSD test, $P < 0.05$.

Greenhouse experiment. In 2019, elderberry plants were treated similarly to the 2018 experiment. However, injury symptoms were observed on all herbicide-treated and nontreated plants following herbicide drift from an adjacent property, so the study was abandoned. Thus, an experiment was conducted in a greenhouse located at the University of Missouri, Columbia, MO, where there was minimal risk of unintended herbicide injury in 2020. Two-year-old American elderberry plants were obtained from the same nursery source as described above on 5 May 2020. Plants were transplanted, pruned, and fertilized as in the previous experiment. On 27 May 2020, herbicide treatments were applied to non-flowering plants and those at the TGC stage. Herbicide treatments included dicamba or 2,4-D at the 1/20 and 1/200 rate. Similar treatments were applied to plants at FB on 12 June 2020. Nontreated control plants were also included for comparison. Herbicides were applied outdoors as previously described and plants from each herbicide treatment were isolated in separate greenhouses for 72 h without irrigation. Plants were then arranged in a randomized complete block design with five replications of each treatment in a greenhouse. The greenhouse was maintained at 26 °C day/20 °C night cycle under natural light for duration of the study and plants were hand-watered as needed.

Cane length data from the V and TGC stages were first subjected to a one-way ANOVA to determine if herbicide treatments differed from nontreated controls. Next, cane length data for nontreated controls were deleted and the remaining data were analyzed as a factorial arrangement of treatments (2 herbicide treatments x 2 herbicide rates x 2 growth stages).

Fruit and juice data from the TGC stage were subjected to a one-way analysis of variance since umbel abortion occurred for one herbicide/treatment combination. Fruit and juice data from the FB stage were first analyzed as a one-way ANOVA to determine if herbicide treatments differed from the nontreated controls. Next, fruit and juice data for the control were deleted and the remaining data were analyzed as a factorial arrangement of treatments (2 herbicide treatments x 2 herbicide rates). All data were analyzed using the PROC GLIMMIX statement in SAS (SAS Institute, Cary, NC). Means for cane length of plants selected at V and TGC stages, and fruit and juice characteristics from canes selected at TGC were separated by Fisher's protected least significant difference (LSD) test, $P < 0.05$. Differences in means for fruit and juice characteristics from canes selected at FB stage were based on a one degree of freedom F-test.

Juice from each treatment and rate for TGC and FB stages were extracted for herbicide residue analysis and pooled by plant. Due to the limited amount of juice, three replications of each treatment and rate were analyzed in 2020. For each sample, 40 mL juice of each treatment was then frozen at -22 °C and sent by overnight mail to South Dakota Agricultural Laboratories (Brookings, SD). Herbicide 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 5 ppb. Because plants treated with one herbicide/rate combination at TGC did not produce fruit, herbicide residue data from juice samples of plants sprayed at the TGC stage were analyzed as a one-way ANOVA. For the FB stage, herbicide residue treatments were factorially arranged (2 herbicides x 2 stages of growth) due to the absence of herbicide residue in the nontreated control juice. Residue data were analyzed using the PROC GLIMMIX statement in SAS (SAS Institute, Cary, NC).

Results

Outdoor Nursery Experiment

Plant injury symptoms for V and TGC stage. Both herbicides induced epinasty at the stem apex and downward twisting of adjacent leaf petioles on plants by 2 WAT. Also, 2,4-D and dicamba treatments caused tissue splitting near the stem apex and the development of adventitious root initials on the main stems and peduncles of the inflorescence (Fig. 1). For the V stage, three canes each on plants treated with 2,4-D or dicamba exhibited necrosis at the terminal growing point by 4 WAT.

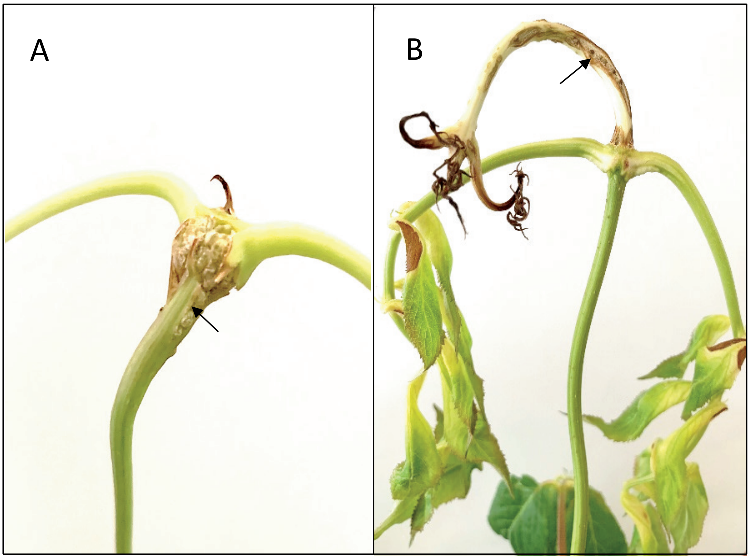

Fig. 1. Elderberry plants. (A) Necrotic stem apex of an elderberry plant treated at a vegetative stage with 2,4-D at 1/20 the labeled rate and (B) necrotic umbel with split peduncle tissue on a plant treated at the tight green cluster stage with dicamba at 1/20 the labeled rate at four weeks after treatment. Adventitious root initials (arrow) emerging from split stem tissue.

Fig. 2. Elderberry flowers treated at the tight green cluster stage with (A) 2,4-D, (B) dicamba, or (C) nontreated weeks after treatment. Herbicides were applied at 1/20 the labeled rate.

For the TGC stage, 2,4-D and dicamba caused umbel abortion on 10 and 8 canes, respectively. For plants treated with 2,4-D at TCG that did not have an aborted umbel, flowers often had distorted petals and stunted filaments compared with those on nontreated control plants (Fig. 2). On plants sprayed with dicamba at TGC that did not have an aborted inflorescence, flowers had undersized petals and necrotic, flattened filaments that were reflexed.

Cane growth for V and TGC stage. By 4 WAT, one cane on a plant treated with 2,4-D at the V stage exhibited necrosis at the stem apex. Three canes on plants treated with dicamba at the V stage averaged 2 cm dieback, resulting in stimulated axillary shoot growth. By 8 WAT, dieback on ten canes treated with dicamba ranged from 1 to 6 cm-long. The treatment-by-stage interaction for cane length was significant (Table 1) by 8 WAT. Plants treated with 2,4-D and dicamba at the V stage reduced cane length by 42 and 29%, respectively, compared with the nontreated control (Table 1). For the TGC stage, plants treated with 2,4-D or dicamba increased cane length compared with nontreated control plants. Also, nontreated and herbicide-treated plants at the TGC stage had less cane growth than the comparable treatment at the V stage.

Fruiting for V and TGC stage. Only three of 20 canes on nontreated plants selected at V

Table 1. Outdoor nursery study. Increase in cane length of elderberry plants treated at a vegetative (V) or tight green flower cluster (TGC) stage of growth with 2,4-D or dicamba at 8 WAT.[z]

Herbicide treatment	Cane length (cm)	
	Stage at treatment	
	V	TGC
2,4-D	9.2 Ba	5.2 Ab
Dicamba	11.3 Ba	4.3 Ab
Control	15.9 Aa	1.6 Bb
Significance	*P* value	
Herbicide (H)	0.6564	
Stage (S)	<0.0001	
H x S	0.0438	

[z] Herbicides were applied at 1/20 of the labeled rate (dicamba 0.56 kg ae·ha^{-1} or 2,4-D 1.06 kg ae·ha^{-1}). WAT=weeks after treatment. Values represent the mean of 5 replications of each treatment. 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, according to Fisher's protected LSD test ($P \leq 0.05$).

Table 2. Outdoor nursery study. Elderberry fruit and juice characteristics from canes treated with herbicide at tight green flower cluster stage of growth on 1 June 2018.[z]

Herbicide treatment	Fruit wt./cane (g)	Drupe no./ cane	Mean drupe wt. (mg)	Juice/ umbel (ml)	Juice/ drupe (µL)
2,4-D	20.8 b	202 b	103 a	7.6 b	38 b
Dicamba	5.7 c	66 c	86 b	2.0 b	30 b
Control	48.7 a	508 a	96 a	25.6 a	50 a

[z] Herbicides were applied at 1/20 of the labeled rate (dicamba 0.56 kg ae·ha^{-1} or 2,4-D 1.09 kg ae·ha^{-1}). Means within a column followed by the same letter are not significantly different, according to Fisher's protected LSD test ($P \leq 0.05$).

stage produced fruit. For these canes, mean fruit weight/cane, drupe number/cane, and drupe weight was 57.2 g, 615 drupes, and 93 mg, respectively. Juice/umbel averaged 30.7 mL and juice/drupe averaged 50 µL for the three nontreated control canes for the V stage. Floral development did not occur on plants treated with either herbicide at the V stage.

Fruit was harvested on 10 and 12 canes of plants treated with 2,4-D or dicamba, respectively, and 20 canes on nontreated controls at the TGC stage. Both herbicides reduced fruit weight/cane and drupe number/cane compared with the nontreated control (Table 2). However, dicamba reduced fruit weight/cane by 88% and drupe number/cane by 87% compared with the nontreated control, whereas 2,4-D reduced fruit weight/cane by 57% and drupe number/cane by 60%. Only dicamba adversely affected mean drupe weight (Table 2). Plants treated with either herbicide produced less juice/umbel (70 to 92%) and juice/drupe (24 to 39%) than the nontreated controls (Table 2).

Plant injury symptoms for FB stage. Injury symptoms were similar to those of plants treated at the TGC stage but appeared less pronounced. No visible injury was observed on nontreated control plants.

Fruiting for FB stage. Canes of all plants treated at FB produced fruit. By harvest, 2,4-D and dicamba treatments reduced fruit weight/cane by 73 and 79%, respectively, compared with the nontreated control (Table 3). Dicamba-treated plants produced fewer drupes/cane than 2,4-D-treated plants (Table 3). However, both herbicides reduced mean drupe weight (8%), juice/umbel (78 to 84%), and juice/drupe (22 to 31%) similarly compared with the nontreated control (Table 3). Soluble solid content, pH, TA, and SSC/TA of juice were similar among all treatments (Table 3).

Greenhouse Experiment

Plant injury symptoms for V and TGC

Table 3. Outdoor nursery study. Fruiting characteristics of elderberry plants treated at full bloom with 2,4-D or dicamba on 22 June 2018.[z]

Herbicide treatment	Fruit wt./cane (g)	Drupe no./ cane	Mean drupe wt. (mg)	Juice/ umbel (ml)	Juice/ drupe (µL)	SSC (°Brix)	pH	TA (g·100 mL^{-1})	SSC/TA
2,4-D	14.6 b	175 b	83 b	6.6 b	38 b	9.0 a	4.9 a	0.29 a	31.0 a
Dicamba	11.6 c	140 c	83 b	4.7 b	34 b	8.7 a	4.9 a	0.32 a	27.2 a
Control	54.4 a	604 a	90 a	29.6 a	49 a	9.7 a	4.9 a	0.34 a	28.5 a

[z] Herbicides were applied at 1/20 the labeled rate (dicamba, 0.56 kg ae·ha^{-1} or 2,4-D, 1.06 kg ae·ha^{-1}). SSC = soluble solid content and TA = titratable acidity expressed as citric acid. Values represent the mean of 5 replications of each treatment. Means within a column followed by the same letter are not significantly different, according to Fisher's protected LSD test ($P \leq 0.05$).

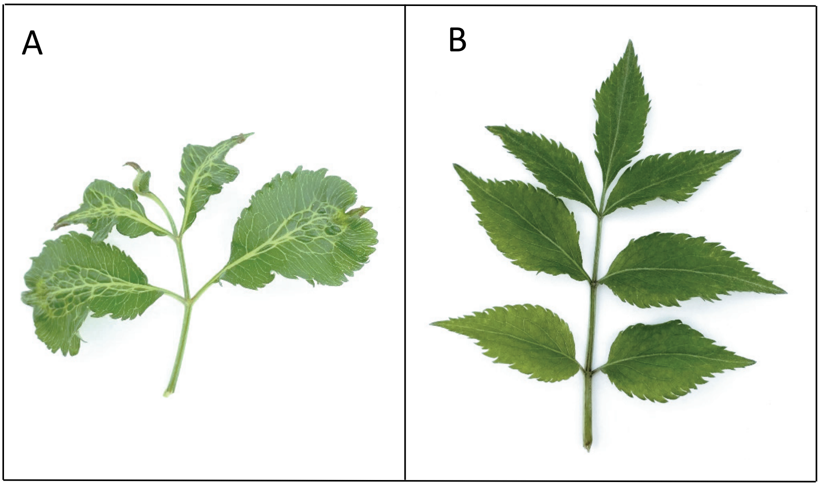

Fig. 3. (A) Obovate leaflets with distorted venation from an elderberry plant treated at the vegetative stage with dicamba at 1/200 the labeled rate and (B) nontreated control leaflets at four weeks after treatment.

stage. Injury symptoms of plants treated at the V or TGC stage with an auxin herbicide at the 1/20 rate in this experiment were similar to those in the nursery study. For the 1/200 rate, obovate leaflets with parallel venation were visible near the distal portions of shoots of plants treated at the V stage with dicamba in the greenhouse experiment (Fig. 3). Leaflets from plants treated at the V stage with 2,4-D at the 1/200 rate were similar to those from untreated controls. Flowers on plants treated at TGC with 2,4-D at the 1/200 rate, were small with stunted filaments, whereas those from dicamba-treated plants at the same rate had reflexed petals with recurved, elongated filaments (Fig. 4).

Cane growth for V and TGC stage. None of the nontreated control plants exhibited herbicide injury during this experiment. For plants treated at the V stage, necrosis was visible at the terminal growing point of twelve 2,4-D- and four dicamba-treated canes at the 1/20 rate but necrosis was not observed on any herbicide-treated plant at the 1/200 rate by 4 WAT.

For the V stage, the mean increase in cane length for nontreated control plants was 11 to 35% greater than that for all herbicide-treated plants, except for 2,4-D-treated plants at the 1/200 rate at 8 WAT ($P \leq 0.0001$). For

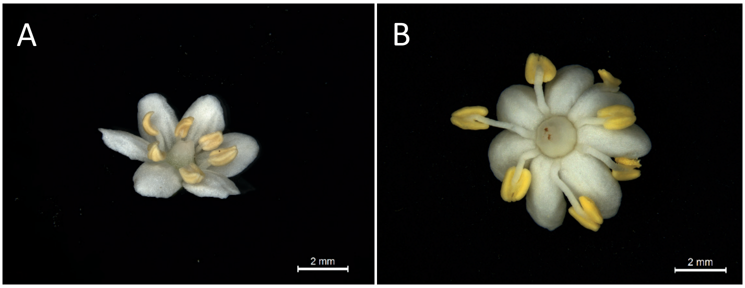

Fig. 4. Elderberry flowers treated at the tight green cluster stage with (A) 2,4-D or (B) dicamba two weeks after treatment. Herbicides were applied at 1/200 the labeled rate.

Table 4. Greenhouse study. Increase in cane length at 8 WAT of elderberry plants treated at a vegetative (V) or tight green flower cluster (TGC) stage of growth with 2,4-D or dicamba on 27 May 2020.[z]

Herbicide treatment	Herbicide rate	Cane length (cm) Stage at treatment V	 TGC
2,4-D	1/20	21.1 Da	13.0 Cb
2,4-D	1/200	35.1 Aa	14.8 Bb
Dicamba	1/20	26.2 Ca	11.8 Cb
Dicamba	1/200	29.0 Ba	17.6 Ab
Control		32.5	14.0
Significance		*P* values	
Herbicide (H)		0.5384	
Rate (R)		<0.0001	
Stage (S)		<0.0001	
H x R		<0.0001	
H x S		0.0136	
R x S		<0.0001	
H x R x S		<0.0001	

[z] Herbicides were applied at 1/20 or 1/200 the labeled rate (dicamba, 0.56 kg ae·ha^{-1} or 2,4-D, 1.06 kg ae·ha^{-1}). Values represent the mean of 5 replications of each treatment. Means for each herbicide and rate within a column followed by the same uppercase letters and means within a row followed by the same lowercase letters are not significantly different by Fisher's protected least significant difference test, $P \leq 0.05$.

the TGC stage, plants treated with dicamba at the 1/200 rate had a greater increase in cane length (26%) than nontreated controls and those treated with 2,4-D at the 1/200 rate had similar cane growth compared with nontreated controls.

The three-way interaction was significant for increase in cane length when data were analyzed as a factorial experiment (Table 4). For each herbicide/rate combination, the increase in cane length after herbicide application was always greater at the V stage than the comparable treatment at the TGC stage. For the V stage, plants treated with 2,4-D at the 1/200 rate produced the greatest cane growth and those treated with the same herbicide at the 1/20 rate had the least growth. For the TGC stage, plants treated with dicamba at the 1/200 rate had the greatest increase in cane length, whereas both herbicides at the 1/20 rate had the least cane growth.

Fruiting for V and TGC stage. For the V stage, none of the canes on herbicide-treated plants developed flowers and only one nontreated control cane produced flowers and fruit. Also, all plants at TGC treated with 2,4-D at the 1/20 rate had 100% flower abortion in umbels and two canes of plants treated with dicamba at the 1/20 rate failed to produce fruit. For the TGC stage, all other canes, regardless of the treatment or herbicide rate, produced fruit.

For plants that fruited, those sprayed with either herbicide at the 1/200 rate produced similar fruit weight/cane as untreated plants (Table 5). However, fruit weight/cane was reduced by 2,4-D at the 1/200 rate (11%) and dicamba at the 1/20 rate (93%) compared with dicamba at the 1/200 rate. Similarly, plants treated at TGC with dicamba at 1/20

Table 5. Greenhouse study. Fruit and juice characteristics from drupes of elderberry plants treated with herbicide at tight green flower cluster stage of growth with 2,4-D or dicamba on 27 May 2020.[z]

Herbicide treatment	Herbicide rate	Fruit wt./cane (g)	Drupe no./ cane	Mean drupe wt. (mg)	Juice/ umbel (ml)	Juice/ drupe (µL)
2,4-D	1/20[y]	---	---	---	---	---
2,4-D	1/200	52.5 b	497 b	106 a	28.7 a	57.7 a
Dicamba	1/20	4.0 c	58 c	69 c	2.1 b	36.2 c
Dicamba	1/200	58.7 a	604 a	97 b	30.3 a	50.2 b
Control	---	55.8 ab	497 b	112 a	27.9 a	56.2 ab

[z] Herbicides were applied at 1/20 or 1/200 the labeled rate (dicamba, 0.56 kg ae·ha^{-1} or 2,4-D, 1.06 kg ae·ha^{-1}). Values represent the mean of 5 replications of each treatment. Means within a column followed by the same letter are not significantly different, according to Fisher's protected LSD test ($P \leq 0.05$).

[y] 2,4-D at the 1/20 rate caused umbel abortion on all canes.

rate had the lowest drupe number/cane and mean drupe weight among all treatments (Table 5). Dicamba at the 1/20 rate was the only treatment at TGC that reduced mean juice/umbel (93%) and juice/drupe (36%) compared with the nontreated control.

For the TGC stage, SSC in juice was the highest from nontreated control plants, intermediate from dicamba-treated plants, and lowest from the 2,4-D-treated plants (Table 6). Although pH and TA of juice were similar among all treatments, SSC/TA from all herbicide-treated plants was lower than that from nontreated controls (Table 6). Residues from herbicides were detected in elderberry juice even though exposure occurred 50 to 69 d before the fruit was harvested for juice extraction. Juice from plants treated with dicamba at the 1/20 rate had the highest herbicide residue content, while residue in juice from 2,4-D treated plants at the 1/200 rate was intermediate, and that from dicamba at the 1/200 rate was the lowest (Table 6).

Fruiting for FB stage. By 4 WAT, 100% floral abortion occurred on four 2,4-D canes of plants treated at the 1/20 rate and one cane of plants treated with dicamba at the same rate. Nontreated control plants at the FB stage had greater fruit weight/cane and a higher number of drupes/cane than any of the herbicide-treated plants ($P < 0.0001$) (Table 7). For the factorial analyses, the two-way interactions of all fruit and juice characteristics were significant, except TA and SSC/TA (Tables 7 and 8). For the 1/20 rate, 2,4-D-treated plants produced more fruit weight/cane and a higher number of drupes/cane than dicamba-treated plants (Table 7). In contrast, 2,4-D-treated plants produced less fruit weight/cane and a lower number of drupes/cane compared with dicamba-treated plants at the 1/200 rate. For all fruit and juice characteristics presented in Table 7, each herbicide applied at the 1/20 rate had a greater negative impact than the same treatment at the 1/200 rate.

Nontreated control plants at the FB stage had similar mean drupe weight compared with those treated with 2,4-D at the 1/200 rate ($P < 0.1458$) (Table 7). In the factorial analysis, 2,4-D-treated plants had less mean drupe weight compared with dicamba-treated plants at the 1/20 rate. In contrast, 2,4-D-treated plants had greater mean drupe weight than dicamba-treated plants at the 1/200 rate.

Nontreated control plants produced 21 to 84% more juice/umbel than that produced by all herbicide-treated plants (P <0.0001) (Table 7). Plants treated with 2,4-D produced more juice/umbel than those treated with dicamba at the 1/20 rate, whereas herbicide-treated plants produced similar amounts of juice at the 1/200 rate.

Table 6. Greenhouse study. Elderberry juice characteristics from canes treated with herbicide at the tight green cluster stage of growth on 27 May 2020. ^{z}

Herbicide treatment	Herbicide rate	SSC (°Brix)	pH	TA (g·100 mL^{-1})	SSC/TA	Herbicide residue (ppb)^{y}
2,4-D	1/20^{x}	---	---	---	---	---
2,4-D	1/200	8.3 c	4.56 a	0.44 a	18.9 b	65 b
Dicamba	1/20	8.9 b	4.60 a	0.43 a	20.7 b	159 a
Dicamba	1/200	8.9 b	4.51 a	0.43 a	20.7 b	12 c
Control	---	10.0 a	4.64 a	0.35 a	28.6 a	ND^{x}

^{z} Herbicides were applied at 1/20 and 1/200 of the labeled rate (dicamba 0.56 kg ae·ha^{-1} or 2,4-D 1.06 kg ae·ha^{-1}). SSC = soluble solid content and TA=titratable acidity expressed as citric acid . Values represent 5 replications of each treatment. Means within a column followed by the same letter are not significantly different, according to Fisher's protected LSD test ($P \leq 0.05$).

^{y} Values represent the mean of 3 replications of each treatment. ND = no herbicide residue detected.

^{x} 2,4-D at the 1/20 rate caused umbel abortion on all canes.

Table 7. Greenhouse study. Elderberry fruit and juice characteristics from canes treated with herbicide at the full bloom stage of growth on 12 June 2020. ^{z}

Herbicide treatment	Fruit wt./cane (g)		Drupe no./cane		Mean drupe wt. (mg)		Juice/umbel (ml)		Juice/drupe (µL)	
	Herbicide rate		Herbicide rate		Herbicide rate		Herbicide rate		Herbicide rate	
	1/20	1/200	1/20	1/200	1/20	1/200	1/20	1/200	1/20	1/200
2,4-D	16.4 Ab	37.8 Ba	181 Ab	334 Ba	90 Bb	113 Aa	6.1 Ab	18.5 Aa	33.6 Ab	55.3 Aa
Dicamba	12.0 Bb	42.5 Aa	126 Bb	391 Aa	95 Ab	109 Ba	3.7 Bb	18.0 Aa	29.8 Bb	46.0 Ba
Control	52.5		456		115		23.3		51.3	
Significance	*P* values									
Herbicide (H)	0.7932		0.6321		0.8201		0.0029		<0.0001	
Rate (R)	<0.0001		<0.0001		<0.0001		<0.0001		<0.0001	
H x R	<0.0001		<0.0001		0.0001		0.0321		0.0198	

^{z} Herbicides were applied at 1/20 and 1/200 of the labeled rate (dicamba 0.56 kg ae·ha^{-1} or 2,4-D 1.06 kg ae·ha^{-1}). Values represent the mean of 5 replications of each treatment. 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.

Plants treated with 2,4-D at the 1/200 rate produced more juice/drupe than nontreated controls ($P < 0.0001$) (Table 7). However, plants of all other herbicide treatments produced less juice/drupe (10 to 42%) compared with the nontreated control. For both herbicide rates, 2,4-D-treated plants produced more juice/drupe than dicamba-treated plants.

Fruit from nontreated control plants had a higher SSC than that from all herbicide-treated plants ($P < 0.0001$) (Table 8). Fruit from dicamba-treated plants had a higher SSC than fruit from 2,4-D-treated plants at the 1/20 rate but SSC of fruit from herbicides were similar at the 1/200 rate.

Fruit from plants treated with dicamba at the 1/20 rate had a lower pH (4.70) than that from all other herbicide-treated plants, but none of the pH values differed from the nontreated control (pH=5.04) ($P = 0.0184$). In the factorial analysis, the main effects of herbicide and rate were significant at $P =$

0.0137 and $P = 0.0528$, respectively, but the interaction was nonsignificant ($P = 0.5237$). Among herbicide treatments, fruit from 2,4-D-treated plants had a higher pH (5.28) than that from dicamba-treated plants (4.89). Among herbicide rates, plants treated at the 1/200 rate had higher pH (5.23) than those treated at the 1/20 rate (4.94).

Titratable acidity of fruit was similar among all treatments including the nontreated control ($P = 0.1168$) and there were also no significant differences in TA of fruit in the factorial analysis (Table 8). However, fruit from nontreated control plants had a higher SSC/TA ratio than all herbicide treatments ($P = 0.0020$). None of the effects were significant for SSC/TA ratio in the factorial analysis (Table 8).

Herbicide residue was detected in the juice of all samples from2,4-D and dicamba-treated plants (Table 8). For the 1/20 rate, juice from 2,4-D-treated plants had a higher herbicide residue content than that from dicamba-treated plants, but the residues were similar for the 1/200 rate.

Discussion

Results from this study demonstrated that elderberry plants are sensitive to low-dose applications of 2,4-D and dicamba. Although both auxin herbicides caused vegetative and reproductive injury to elderberry, the type and degree of injury varied by the stage of plant growth and herbicide rate at which plants were treated. When plants were treated at the V stage (i.e., before any flowers were visible), both herbicides at the 1/20 rate caused necrosis of the terminal growing point and/or cane dieback in both experiments. Dicamba at the 1/200 rate had no effect on cane growth when applied at the V stage, but 2,4-D applied at this rate had 8% more growth compared with the nontreated control by 8 WAT. This may be the result of hormesis, in which an inhibitor has a stimulatory effect at a low dose. Herbicide-induced hormesis or growth stimulation at low rates of application has been reported by others (Appleby 1998; Cedergreen, 2008; Dintelmann et al., 2019; Joseph and Peter, 1980).

In an earlier study, Dintelmann et al. (2019) reported that elderberry plants treated at a non-flowering stage with 2,4-D or dicamba at a 1/20 rate had 12 or 20% estimated visible injury and plants treated with either herbicide at 1/200 rate had only 1% injury 56 d after treatment. In the same study, cane lengths of herbicide-treated plants at both rates were

Table 8. Greenhouse study. Elderberry juice characteristics from fruit on canes treated with herbicide at the full bloom stage of growth on 12 June 2020.[z]

Herbicide treatment	SSC (°Brix) Herbicide rate		TA (g·100 mL^{-1}) Herbicide rate		SSC/TA Herbicide rate		Herbicide residue (ppb) Herbicide rate	
	1/20	1/200	1/20	1/200	1/20	1/200	1/20	1/200
2,4-D	7.6 Ba	7.7 Aa	0.39 Aa	0.40 Aa	19.5 Aa	19.3 Aa	391 Aa	88 Ab
Dicamba	8.1 Aa	7.6 Ab	0.49 Aa	0.37 Aa	16.5 Aa	20.5 Aa	41 Ba	15 Ab
Control	9.9		0.33		30.0		ND[y]	
Significance	*P* values							
Herbicide (H)	0.0810		0.4383		0.9994		<0.0001	
Rate (R)	0.0080		0.2650		0.2861		<0.0001	
H x R	0.0005		0.1677		0.1447		<0.0001	

[z] Herbicides were applied at 1/20 and 1/200 of the labeled rate (dicamba 0.56 kg ae·ha^{-1} or 2,4-D 1.06 kg ae·ha^{-1}). SSC = soluble solid content and TA = titratable acidity expressed as citric acid. 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.

[y] ND = none detected.

similar to that of nontreated control plants 112 d after application when only the main cane was measured. However, in the present study, the main cane, as well as subsequent axillary cane growth was measured, which more accurately reflects the effect of the herbicide on whole-plant growth.

The reproductive consequence of auxin herbicide exposure at a non-flowering (V) stage of elderberry plants was difficult to determine in our study. Although none of the plants treated with an herbicide at the V stage produced flowers, only 3 of 20 nontreated control canes in the nursery study and 1 control cane in the greenhouse trial produced umbels after cane selection. Thus, it may be possible that many of the canes selected for treatment at the V stage were incapable of differentiating floral primordia. Because floral differentiation occurs on current season's growth from one- and two-year-old canes with about 8 or more leaves, the selection of canes that will later develop flowers can be problematic early in the growing season when herbicide treatments were applied (Charlebois et al., 2010).

Elderberry canes produced little growth after the TGC stage. Also, the effect of herbicides applied at the 1/20 rate at the TGC stage differed in the two experiments. Plants treated with herbicides at the 1/20 rate had greater cane growth compared with the nontreated control canes in the nursery study, but it was suppressed by herbicides in the greenhouse (Tables 1 and 4). Additionally, in the nursery study, where there was little increase in vegetative growth, cane elongation and slight epinasty occurred on the main cane just below the inflorescence. In contrast, cane elongation occurred below the umbel (with considerable epinasty) and from newly-developed axillary growth when plants were grown in the greenhouse. Differences in herbicide responses may be associated with ambient environmental conditions at the time of treatment or the growing environment shortly after applications. On the date of treatment, the ambient air temperature was 22 °C with heavy cloud cover for the nursery study versus 25 °C and no cloud cover for the greenhouse experiment, which likely increased the absorption and activity of herbicides when applied at the higher temperature (Ganie et al., 2017).

Both herbicides at the 1/20 rate caused severe floral distortion or complete (100%) abortion in umbels on canes when applied at the TGC stage. For the canes that produced drupes, fruit weight/cane and juice/umbel were reduced by both herbicides at the 1/20 rate (Tables 2 and 5). In contrast, herbicides applied at the lower dosage had no effect on fruit weight/cane and juice/umbel compared with the nontreated control (Table 5). However, when plants were treated at TGC with 2,4-D at the 1/200 rate (no fruit produced on plants at the 1/20 rate) or dicamba at both rates, SSC and SSC/TA ratios of juice were less than those in juice from nontreated controls (Table 6). The lower SSC caused by the herbicide would likely negatively impact juice processors due to the additional cost associated with the need for increased use of sweeteners. Because SSC of elderberry juice is naturally low (~ 9.7 to 13.1 °Brix), processors usually add high concentrations of sweeteners to their products, such as sucrose, sorghum syrup, agave nectar, or pear juice to as much as 47 °Brix (Thomas et al., 2013; Warmund et al., 2016).

Complete floral abortion in umbels occurred on $\leq$ 20% of the canes treated at FB with either herbicide at the 1/20 rate, but complete abortion was not observed on any of the canes treated with either herbicide at the 1/200 rate. For the canes treated at the higher rate, herbicides reduced fruit weight/cane and juice/umbel compared with the nontreated controls, but juice/umbel was similar for plants treated with either herbicide at the 1/200 rate. Neither herbicide rate had a consistent negative effect of juice SS, pH, TA, or SSC/TA when applied at FB (Tables 3 and 8). Collectively, results from this study indicate that elderberry plants were most sensitive to cane injury and floral abortion when treated with an auxin herbicide at TGC versus the FB stage. Similar results have been reported for herbaceous plants. Greater yield losses were reported for tomato when low rates of synthetic auxin herbicides were applied at early bloom compared with a non-flowering or fruit set stage (Fagliari et al., 2005; Kruger et al., 2012). Herbicide-sensitive soybeans suffered a greater yield loss when dicamba drift occurred at the RI stage (beginning bloom) than at later stages (Griffin et al., 2013; McCown et al., 2018). After the R5 stage (seed filling in pods), low rates of dicamba had no significant effect on visible injury, plant height, or yield (Scholtes, 2014).

Elderberry plants were generally more sensitive to complete floral abortion in umbels when treated with 2,4-D than dicamba at the 1/20 rate, especially when exposure occurred at TGC. Plants treated at TGC with 2,4-D at the 1/20 rate had complete abortion on 50 to 100% of the canes treated, whereas dicamba caused complete abortion on 10 to 40% of the canes in the two experiments. At FB, 2,4-D at the 1/20 rate caused complete floral mortality on 0 to 20% of the treated canes and dicamba caused complete floral mortality on 0 to 5% of canes.

Herbicide residue was detected in juice from all fruit-bearing plants when treated at either developmental stage, ranging from 12 to 391 ppb for both greenhouse experiments (Tables 6 and 8). However, for the 1/200 rate, juice samples from 2,4-D treatments contained more than five times more residue than juice from dicamba treatments in both experiments. According to the Environmental Protection Agency (EPA), the maximum legal 2,4-D residue limit (i.e., tolerance) for berry crops group 13, which includes elderberry, is 200 ppb (Office of Federal Register, 2021). Thus, harvested fruit exposed to 2,4-D at the 1/20 rate at FB would trigger enforcement action by EPA and would be subject to seizure by the United States government (US EPA 2020). To date, a dicamba tolerance for fruit crops has not been established (Office of Federal Register, 2021). However, dicamba residue tolerance for asparagus, soybean seed, and milk are 4,000, 10,000, and 200 ppb, respectively. The highest average residue content (159 ppb) was detected in juice from harvested fruit on plants treated at TCG with dicamba at the 1/20 rate. However, pesticides in fruits decrease considerably with exposure to light, washing, and cooking (US EPA, 2020).

In conclusion, elderberry plants are sensitive to low dose applications of 2,4-D and dicamba, which can cause reproductive losses and a reduced SSC, especially when exposure occurs during the TGC stage. In Missouri, TGC and bloom stages of elderberry occur from late-May to early June, which coincides with the postemergence applications of dicamba or 2,4-D to transgenic soybeans. Thus, the risk of off-target herbicide movement and subsequent damage to elderberry may be substantive in areas where transgenic soybean is grown nearby. Further elderberry losses can occur if herbicide residues in fruit juice exceed the allowable amount regulated by EPA. Until dicamba residue tolerance levels are established by EPA for elderberry, the risk of producing fruit that may later be subject to crop destruction cannot be determined.

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