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Effect of Glyphosate and Dicamba Drift Timing and Rates in Bell Pepper and Yellow Squash

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As dicamba resistance traits become more common in agronomic crops, the potential for off-site movement also increases. Little is known of how common vegetable crops will respond to dicamba drift. The objective of this study was to evaluate the effect of dicamba and glyphosate drift on bell pepper and squash growth as a function of application timing. The treatments were arranged in a factorial design with two timings by three rates and a nontreated check. The two timings were early bloom and midbloom (during bloom when fruit were present). The three rates were glyphosate at 21 + dicamba at 14 g ha^{-1}, glyphosate at 10 + dicamba at 7 g ha^{-1}, and glyphosate at 7 + dicamba 5 at g ha^{-1}. Herbicides were applied with a controlled droplet applicator calibrated to deliver 2.34 L ha^{-1}. In squash, crop injury was 26 to 31% at 3 DAT and 48 to 65% at 17 DAT. However, no differences were measured among application timings or rates for fruit weight or number at individual harvest or season total. Bell pepper injury ranged between 0 and 8% from 3 to 17 DAT and was not significantly different than the nontreated. However, large, Fancy, marketable, and total bell pepper fruit number were greater in the nontreated than glyphosate at 21 + dicamba 14 at g ha^{-1} and glyphosate at 10 + dicamba at 7 g ha^{-1} both years. The three rates of dicamba + glyphosate had a greater number and weight of cull fruit compared to the number of fruit in the nontreated plots. The cull fruit were shorter with a flattened appearance. Leaving bell pepper fruit on the plants longer may result in small and medium fruit becoming large or Fancy grade bell pepper fruit.

Nomenclature: Glyphosate; dicamba; bell pepper, *Capsicum anuum* L.; squash, *Cucumis melo* L.

Key words: Controlled droplet application, off-target movement, synthetic auxin.

Al volverse más común la resistencia a dicamba en cultivos agronómicos, el potencial de movimiento del herbicida a lugares no deseados se incrementa. Se conoce poco de cómo responderán los cultivos de vegetales a la deriva de dicamba. El objetivo de este estudio fue evaluar el efecto de la deriva de dicamba y glyphosate en el crecimiento del pimiento y la calabaza en función del momento de aplicación. Los tratamientos fueron arreglados en un diseño factorial con dos momentos de aplicación y tres dosis y un testigo sin tratamiento. Los dos momentos de aplicación fueron floración temprana y floración media (durante la floración cuando hubo presencia de frutos). Las tres dosis fueron glyphosate a 21 + dicamba a 14 g ha^{-1}, glyphosate a 10 + dicamba a 7 g ha^{-1}, y glyphosate a 7 + dicamba a 5 g ha^{-1}. Los herbicidas fueron aplicados con una aplicador con gota controlada y calibrado para liberar 2.34 L ha^{-1}. En la calabaza, el daño al cultivo fue 26 a 31% a 3 DAT y 48 a 65% a 17 DAT. Sin embargo, no se midieron diferencias entre momentos de aplicación o dosis para el peso o número de frutos para cosechas individuales o para el total de la temporada. El daño en el pimiento varió entre 0 y 8% entre 3 y 17 DAT, y no fue significativamente diferente del testigo sin tratamiento. Sin embargo, el número de frutos grandes, Fancy, comercializables, y totales fue mayor en el testigo que con glyphosate a 21 + dicamba a 14 g ha^{-1} y glyphosate a 10 + dicamba a 7 g ha^{-1} en ambos años. Las tres dosis de dicamba + glyphosate tuvieron un mayor número y peso de fruto de rechazo al compararse con el número de frutos en las parcelas testigo. La fruta de rechazo fue más corta con una apariencia aplanada. El dejar el fruto del pimiento en las plantas por más tiempo podría hacer que frutos pequeños y medianos alcancen un tamaño grande o una categoría Fancy.

As glyphosate-resistant weeds have become more problematic, additional herbicide tolerance genetic traits have been included in agronomic crops. The most recent are soybean and cotton cultivars that are resistant to synthetic auxin herbicides such as 2,4-D and dicamba (Behrens et al. 2007; Braxton et al. 2010; Johnson et al. 2010). However, many horticultural crops are sensitive to auxin herbicide drift (Colquhoun 2014; Flessner 2012; Gilreath et al. 2001a–c). In Florida, vegetable fields and homeowner gardens are commonly grown in close proximity to agronomic fields. Florida's bell pepper

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industry had cash receipts of $164 million (second in the United States) and squash was $41 million (first in the United States) (U.S. Department of Agriculture–National Agricultural Statistics Service [USDA-NASS] 2015).

Previous research demonstrated that dicamba at sublethal rates of 0.11, 1.12, and 11.2 g ha^{-1} applied prior to pepper bloom or at bloom decreased plant vigor, flower number, and yield (Gilreath et al. 2001a). Dicamba at sublethal rates of 60 to 100 g ha^{-1} just prior to tomato bloom of the first cluster and during bloom had greater injury, greater flower abscission, and lower tomato yields compared to exposure at postbloom (Gilreath et al. 2001b). In both pepper and tomato, the decrease in yield was due to the decrease in the number of flowers and fruit set (Gilreath et al 2001a,b).

In cucumber (*Cucumis sativus* L.), 2,4-D at sublethal rates applied at earlier stages of the crop had greater epinasty than application at a later stage; however, there were no differences between application timings for yield (Gilreath et al. 2001c). Application of 2,4-D after the first harvest decreased yield of the second harvest, but yield at the third harvest was similar to the control (Hemphill and Montgomery 1981).

A significant number of research trials document the impact of auxin herbicide in vegetable crops. The sublethal herbicide rates were applied at standard application volumes (Gilreath 2001a–c; Hemphill and Montgomery 1981), thus forming highly diluted spray solutions. Banks and Schroeder (2002) showed glyphosate applied to sweet corn (*Zea mays* L.) at a carrier volume of 281 L ha^{-1} caused less injury and yield loss than when applied at lower carrier volumes (12, 24, 47, and 94 L ha^{-1}), indicating that concentration of the herbicide in the droplet, not just herbicide rate per hectare, must be taken into consideration. As the previous research on auxin herbicide drift in horticultural crops was conducted at standard application volumes, it is possible that the published literature has incorrectly approximated the level of injury. Additionally, the auxin herbicides applied to transformed cotton or soybean will commonly be applied with glyphosate. Because few papers have documented the impact of glyphosate and auxin drift, combining these two will be important as these herbicide combinations become more common. Therefore, the objective of this study was to determine the effect of dicamba and glyphosate drift at lower application volumes on bell pepper and squash, when applied at two growth stages.

Materials and Methods

Experiments were conducted in 2013 and 2014 at the Plant Science Research and Education Unit, Citra, FL. The soil type was an Arredondo sand (loamy, siliceous, semiactive, hyperthermic Grossarenic Paleudults) with soil pH 7. The treatments were a factorial design with three rates by two timings with a nontreated control. The three rates were glyphosate at 21 g ha^{-1} + dicamba at 14 g ha^{-1}, glyphosate at 10 g ha^{-1} + dicamba at 7 g ha^{-1}, and glyphosate at 7 g ha^{-1} + dicamba at 5 g ha^{-1}. These rates represent drift of glyphosate at 0.84 kg ae ha^{-1} + dicamba at 0.56 kg ha^{-1} applied to an agronomic crop at carrier volumes of either 94, 187, or 280 L ha^{-1}. The two timings were early bloom and midbloom (during bloom when fruit were present and were $\leq$ 2.5 cm). Treatments were applied with a controlled droplet applicator (Ulva+, Micron Sprayers, Ltd.) calibrated to deliver 2.34 L ha^{-1}. Droplet size from this applicator was approximately 100 microns (Micron Group 2014). Applications were made at predawn when wind speed was 0 km h^{-1}, and no spray particle movement occurred. Another reason for the early hour was to prevent convective lifting and movement of the spray particles if the soils were at a higher temperature than the air—as is common at midday. Experimental design was a randomized complete block design with four replications. Plots were one bed and 6.1 m long. Plot row middles were kept weed free with paraquat 0.84 kg ha^{-1}, and clethodim at 0.71 kg ha^{-1} and hand weeding were used in the crop holes (Ozores-Hampton et al. 2014). Fertilizer, drip irrigation, and pest management followed industry standards (Ozores-Hampton et al. 2014).

Squash. Yellow squash 'Enterprise' and 'Liberator III' were direct seeded on April 24, 2013, and March 27, 2014, respectively. Squash were grown on a raised bed covered with polyethylene mulch at 1.8 m between-bed spacing and 45.7 cm in-row spacing. The early-bloom applications were May 23, 2013 and May 2, 2014. The midbloom applications were June 10, 2013 and May 5,

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Figure 1. Shortening of bell pepper fruit after glyphosate + dicamba simulated drift applied at early bloom on the left, compared to the nontreated on the right.

2014. Crop injury was visually assessed at 3, 10, and 17 d after treatment (DAT). Squash harvest began on May 30 and May 6 in 2013 and 2014, respectively. In each year, seven additional harvests occurred at 3- or 4-d intervals; fruit were harvested when they were 7.6 to 12.7 cm long. Harvests that occurred before the midbloom application were included in the total yield.

Bell Pepper. Pepper 'Aristotle' and 'Heritage' were transplanted on April 24, 2013, and March 27, 2014, respectively. Peppers were grown on polyethylene-covered raised beds at 1.8 m between-bed spacing and two rows per bed at 0.3 m between and in-row spacing. The early-bloom applications were May 23, 2013 and May 6, 2014. The midbloom applications were June 17, 2013 and May 22, 2014. At the midbloom application, the plants had five to six flowers and fruit were 1.27 to 5.08 cm in diameter. Crop injury was visually assessed at 3, 10, and 17 d after application. Bell pepper fruit were harvested at horticulture maturity on July 11, 2013 and June 10, 2014. Bell pepper fruit were graded into USDA standard cull, small, medium, large, and Fancy grades (USDA–Agricultural Marketing Service [USDA-AMS] 2005). Cull fruit typically had a compact habit with a smaller length-to-width proportion than fruit in the nontreated control (Figure 1).

Data were tested for normality and analyzed with Proc GLM in SAS (SAS v. 9.2, SAS Institute Inc., Campus Drive, Cary, NC 27513). Means were separated by Fisher's protected LSD ($P \leq 0.05$). Squash yield data were analyzed for individual hares, total yield including all hares, and total yield excluding harvest prior to the midbloom application. Bell pepper yield was analyzed by grade, marketable yield, and total yield.

Table 1. Impact of glyphosate and dicamba simulated drift with controlled droplet applicator applied at early and midbloom on squash injury.

Rate	3 DAT	10 DAT	17 DAT
g ae ha^{-1}		%	
Glyphosate 7 + dicamba 5	22	50 b	51 b
Glyphosate 10 + dicamba 7	26	58 a	60 ab
Glyphosate 21 + dicamba 14	29	55 ab	64 a

[a] The spray solution was applied at 2.34 L ha^{-1} to simulate drift.

[b] Application timing was not significant and means were pooled. Means followed by different letters are significantly different within year and separated with Fisher's protected LSD ($P \leq 0.05$).

Results and Discussion

Squash. The year-by-treatment interaction was not significant for crop injury or yield, so data were pooled across year. The application timing–by–rate interaction was significant for squash injury. All the treated plots had greater crop injury than the nontreated (data not presented). The squash crop injury was 22 to 29% at 3 DAT and 51 to 64% at 17 DAT (Table 1). The crop injury observed at 3 DAT was curling of the new growth, and the older leaves began to wilt and lay flat on the bed surface. At 17 DAT, the observable injury was leaf cupping and chlorosis of the new leaves, whereas some of the older leaves were still lying flat. Individual harvest and yearly total yield were not different among

Table 2. Impact of glyphosate + dicamba simulated drift applied at early and midbloom on bell pepper injury.

Rate[a]	Early bloom			Midbloom		
	3 DAT	10 DAT	17 DAT	3 DAT	10 DAT	17 DAT
$g\ ae\ ha^{-1}$	%					
Glyphosate 7 + dicamba 5	0	6 a	3 a	0	4 b	0
Glyphosate 10 + dicamba 7	0	8 a	5 a	0	3 b	0
Glyphosate 21 + dicamba 14	0	0 b[b]	0 b	0	0 a	0
Nontreated	0	0 b	0 b	0	0 a	0

[a] The spray solution was applied at 2.34 L ha^{-1} to simulate drift.

[b] Means followed by different letters are significantly different within year and separated with Fisher's protected LSD ($P \leq 0.05$).

application timings (P values = 0.41 and 0.35, respectively), rate (P values = 0.61 and 0.13, respectively), and timing-by-rate interaction (P values = 0.58 and 0.37, respectively) or rates for fruit weight or number at individual harvest or season total (data not shown). Total squash yield was 37,291 to 42,385 kg ha^{-1} and the total number of fruit were 126,422 to 138,975 fruit ha^{-1}. It was surprising that fruit yield was not impacted, particularly when squash injury of 51 to 64% was observed. This could be attributed to the repeated flowering and fruiting of squash during the growing season. Because both fruit number and size were unaffected, it appears that the squash foliage is more responsive to dicamba and glyphosate residues than floral tissue. Similar results were found with drift rates of aminocyclopyrachlor and dicamba in cantaloupe and cucumber (Flessner et al. 2012; Gilreath 2001c).

Bell Pepper. Year-by-treatment interactions were not significant for bell pepper injury and the data have been pooled. Injury was not observed at 3 DAT after either application timing (Table 2). The injury at 10 and 17 DAT was greater than the nontreated; however, the injury was less than 8%. The injury was subtle and took the form of cupping and crinkling of the new foliage while the stems remained erect with no apparent epinasty.

The year-by-treatment interaction was significant for fruit weight and number, so years have been separated for these variables. Weather both years was similar and typical for Florida. Differences between year may have been cultivar differences or small differences in number of blooms at the time of application. The early bloom application had a greater number of cull fruit both years (Table 3). In 2013, the number of small and medium fruit after the early bloom was similar to the nontreated. Dicamba + glyphosate drift at midfruit had a lower number of fruit than the nontreated. For large and Fancy grades, the nontreated and midbloom applications were similar and the early bloom application had fewer fruit. The large and Fancy grade fruit were present at the time of the

Table 3. Bell pepper fruit number affected by glyphosate + dicamba drift applied with a controlled droplet applicator at two stages of crop plant growth in 2013 and 2014.

Timing	Cull[a]	Small	Medium	Large	Fancy	Total
	Number of fruit ha^{-1} (% of total)					
2013						
Early bloom	14,933 (21) a[b]	13,778 (20) a	11,220 (16) ab	26,153 (38) b	3,383 (5) b	69,465 b
Midbloom	4,455 (5) b	3,300 (4) b	7,425 (8) b	68,360 (73) a	9,488 (10) a	93,027 a
Nontreated	2,970 (3) b	8,168 (8) ab	15,345 (14) a	68,805 (64) a	12,375 (11) a	107,663 a
2014						
Early bloom	88,688 (75) a	825 (1) c	11,385 (10)	7,095 (6) c	10,230 (9) c	118,223 b
Midbloom	1,238 (1) b	8,745 (5) b	12,623 (8)	74,415 (46) b	64,103 (40) a	161,123 a
Nontreated	1,238 (1) b	16,088 (9) a	12,128 (7)	88,358 (51) a	56,035 (32) b	173,845 a

[a] Data were pooled across application rates.

[b] Years are significant and have been separated. Timing * Rate interaction was not significant and rate means were pooled. Means followed by different letters are significantly different within year and separated with Fisher's protected LSD ($P \leq 0.05$).

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Table 4. Bell pepper fruit weight affected by glyphosate + dicamba drift applied with a controlled droplet applicator at two stages of crop plant growth in 2013 and 2014.

Timing	Cull	Small	Medium	Large	Fancy	Total
	kg ha^{-1}					
2013						
Bloom	4,125 (20) a	1,964 (10) a	2,871 (14) ab	9,636 (47) b	1,931 (9) b	20,526 b
Midbloom	1,980 (6) b	611 (2) b	1,997 (6) b	24,024 (72) a	4,620 (14) ab	33,231 a
Nontreated	990 (3) b	1040 (3) ab	3,564 (10) a	23,810 (67) a	6,089 (17) a	35,492 a
2014						
Bloom	32,093 (72) a	116 (0.3) c	4,154 (9)	3,036 (7) c	5,338 (12) c	44,736 b
Midbloom	161 (0.2) b	941 (1) b	2,685 (4)	28,962 (42) b	35,552 (52) a	68,300 a
Nontreated	248 (0.4) b	2,054 (3) a	2,834 (4)	36,519 (56) a	24,008 (37) b	65,662 a

[a] Early bloom application had five to seven flowers plant^{-1} and no fruit present. At the midbloom application, bell pepper plants had five to six flowers and fruit were 1.27 to 5.08 cm in diameter.

[b] Years are significant and have been separated. Timing $*$ Rate interaction was not significant and rate means were pooled. Means followed by different letters are significantly different within year and separated with Fisher's protected LSD ($P \leq 0.05$).

midbloom application, so the herbicide didn't affect fruit present at the time of application. In 2014, the early bloom application had the lowest number of fruit in all fruit grades. The midbloom application had fewer small fruit than the nontreated. Similar trends were seen the fruit weight (Table 4).

Rate of simulated glyphosate and dicamba drift was observed in certain bell pepper grades. For large, Fancy, marketable, and total fruit, the nontreated had a greater number of fruit than glyphosate at 21 + dicamba 14 at g ha^{-1} and glyphosate at 10 + dicamba at 7 g ha^{-1} both years (Table 5). The nontreated was greater than these rates for large fruit in 2013 and marketable and total fruit both years. The three rates of dicamba + glyphosate had a greater number and weight of cull fruit compared to the number of fruit in the nontreated (Tables 5 and 6). In 2013, glyphosate at 7 + dicamba at 5 g ha^{-1} had a greater number of large fruit than the glyphosate at 21 + dicamba at 14 g ha^{-1} application rate and although not significantly different a similar trend was observed for Fancy grade fruit (Table 5). In 2014, the three drift rates were similar for large and Fancy grade bell pepper fruit; however, the lowest drift rate was numerically the highest.

Bell pepper fruit set early in the season results in a greater number of large and Fancy grades; a second set of fruit results in small or medium fruit in a single pick or they can be left on the plants to become larger for large and Fancy grades. This is shown in the nontreated having the greatest number and weight of all the grades resulting in the greatest marketable and total yield. The early bloom

Table 5. Effect of glyphosate + dicamba simulated drift at early and midbloom on bell pepper fruit number in 2013 and 2014.

Rate[a]	Cull	Small	Medium	Large	Fancy	Total
g ae ha^{-1}	Number of fruit ha^{-1} (% of total yield)					
2013						
Glyphosate 7 + dicamba 5	10,890 (12) a	6,930 (8)	10,395 (11)	55,193 (60) b	8,044 (9)	91,451 ab
Glyphosate 10 + dicamba 7	9,158 (11) a	9,158 (11)	10,890 (14)	45,243 (56) bc	5,940 (7)	80,388 b
Glyphosate 21 + dicamba 14	9,034 (13) a[b]	9,529 (13)	6,683 (9)	41,333 (57) c	5,321 (7)	71,899 b
Nontreated	2,970 (3) b	8,168 (8)	15,345 (14)	68,805 (64) a	12,375 (11)	107,663 a
2014						
Glyphosate 7 + dicamba 5	46,283 (30) a	5,198 (3)	12,251 (8)	47,768 (31) b	42,199 (27)	153,698 ab
Glyphosate 10 + dicamba 7	49,748 (36) a	6,311 (5)	7,425 (5)	38,610 (28) b	36,630 (26)	138,724 bc
Glyphosate 21 + dicamba 14	38,858 (31) a	2,846 (2)	16,335 (13)	35,888 (28) b	32,670 (26)	126,596 c
Nontreated	1,238 (1) b	16,088 (10)	12,128 (7)	88,358 (54) a	46,035 (28)	163,845 a

[a] The spray solution was applied at 2.34 L ha^{-1} to simulate drift.

[b] Years are significant and have been separated. Application timing was not significant and means were pooled. Means followed by different letters are significantly different within year and separated with Fisher's protected LSD ($P \leq 0.05$).

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Table 6. Effect of glyphosate + dicamba simulated drift at early and mid-bloom on bell pepper fruit weight in 2013 and 2014.

Rate[a]	Cull	Small	Medium	Large	Fancy	Total
g ae ha^{-1}	kg ha^{-1} (% of total)					
2013						
Glyphosate 7 + dicamba 5	3,069 (10) ab	941 (3)	2,574 (8)	19,676 (65) ab	4,232 (14)	30,492
Glyphosate 10 + dicamba 7	2,426 (9) b	1,584 (6)	2,896 (11)	16,038 (62) b	2,995 (12)	25,938
Glyphosate 21 + dicamba 14	3,663 (15) a[b]	1,337 (6)	1,832 (8)	14,776 (61) b	2,599 (11)	24,206
Nontreated	990 (3) c	1,040 (3)	3,564 (10)	23,810 (65) a	6,089 (17)	35,492
2014						
Glyphosate 7 + dicamba 5	16,137 (26) ab	334 (1)	3,248 (5) ab	18,501 (30)	22,776 (37)	61,287ab
Glyphosate 10+ dicamba 7	18,928 (33) a	625 (1)	1,968 (3) b	15,265 (27)	20,794 (36)	57,578b
Glyphosate 21 + dicamba 14	13,316 (26) b	625 (1)	5,043 (10) a	14,231 (28)	17,764 (35)	50,688c
Nontreated	248 (0.4) c	2,054 (3)	2,834 (4) ab	36,519 (52)	29,223 (42)	69,669a

[a] The spray solution was applied at 2.34 L ha^{-1} to simulate drift.

[b] Years are significant and have been separated. Application timing was not significant and means were pooled. Means followed by different letters are significantly different within year and separated with Fisher's protected LSD ($P \leq 0.05$).

application shifted bell pepper grades from large and Fancy to the less desirable grades of small, medium, and cull. The midbloom application occurred at the time of a second fruit set causing the number of small and medium fruit to be less than the nontreated. Gilreath et al. (2001a) showed the reduction in bell pepper yield was due to the decreased number of flowers. Similarly, our study had a lower number of total fruit, however, the greatest impact on yield was large fruit that were misshapen causing them to be cull fruit. Mohseni-Moghadam and Doohan (2015) reported dicamba at 1.4 to 11.2 g ae ha^{-1} reduced yield at the first harvest; however, total harvest was only different at 2.8 to 11.2 g ae ha^{-1}. Our bell pepper harvest was concentrated to a single harvest, allowing more differences to be measured in bell pepper. Based on the results of these studies, bell pepper growers who observe drift injury should harvest the largest fruit first and leave smaller fruit to stay on the plants to become larger. The floral or fruiting stage at the time of application would be a better estimation of future yield reduction rather than foliar injury. This is indicated by greater foliar injury in squash than bell pepper; however, yield reduction was less in squash than bell pepper.

The use of a controlled droplet applicator for application rates had an effect on the crop depending on the species. In squash, results were similar to results reported in other cucurbit crops that used lower rates of the herbicides at standard application volumes. In bell pepper, Gilreath (2001a) reported no change in cull fruit or fruit shape by dicamba drift. It is unknown if the concentrated droplets may have allowed for more dicamba absorption, or the presence of glyphosate, translated into greater bell pepper yield loss than has been previously observed.

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