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Abstract

The loss of methyl bromide led vegetable growers to rely more heavily on herbicides to control weeds. Although herbicides can be effective, limited options in vegetable production challenge growers. Identifying new, effective tools to be applied over plastic mulch before planting, for improved weed control with minimal crop injury, would be beneficial. The objective of these experiments was to evaluate the persistence of preplant applications of glyphosate (1,125 or 2,250 g ae ha^{-1}) plus 2,4-D (1,065 or 2,130 g ae ha^{-1}) or dicamba (560 g ae ha^{-1}) over plastic mulch, using analytical techniques and subsequent yellow squash and watermelon response. Glyphosate and 2,4-D were not analytically detected at damaging concentrations on plastic mulch when at least 3.5 cm of rainfall was received after application and before planting. In addition, bioassay results showing less than 10% visual injury for either squash and watermelon, with no growth or yield suppression observed, supported analytical results. In contrast, dicamba concentrations on plastic mulch, regardless of rainfall amount or time between application and planting, remained at damaging levels. Squash yields were reduced by dicamba applied 1 to 30 d before planting, whereas watermelon was more resilient. 2,4-D plus glyphosate applied preplant over plastic mulch can provide an additional herbicide option for vegetable growers. More research is needed to understand the impact of residual activity of 2,4-D when transplants land directly in holes in plastic mulch at the time of application. The relationship of dicamba with plastic mulch is complex, because the herbicide cannot be easily removed by rainfall. Thus, dicamba should not be included in a weed management system in plasticulture vegetable production.

Introduction

The loss of methyl bromide from the market place more than a decade ago led to a shift in weed control practices for plasticulture vegetable production systems (Culpepper et al. 2009; Eure and Culpepper 2017; Stevens et al. 2016). Currently, one of the most common vegetable production practices in Georgia is to fumigate using a system comprising 1,3-dichloropropene, chloropicrin, and metam sodium, while covering the bed with either low-density polyethylene mulch or totally impermeable film (TIF) (Culpepper et al. 2008; Culpepper et al. 2017). Exceptional control of many diseases, nematodes, and weeds can be achieved with this fumigant system, especially when applied under TIF (Culpepper et al. 2017). Because of lack of permeability, TIF mulch minimizes the ability of the fumigant to escape the raised bed, improving weed control but also presenting challenges to growers, due to plant-back intervals potentially exceeding 35 d (Culpepper et al. 2017). This, compounded with the impact of environmental conditions on fumigant degradation, has led many growers in Georgia to fumigate their vegetable fields from December to January before planting their crop in March (Csinos et al. 2002; Desaeger et al. 2008). Doing so allows for optimum fumigant activity as well as adequate time for fumigant degradation under the mulched bed. Although this approach provides exceptional pest control under the plastic, with minimal crop injury concerns, weeds in the row middles between plastic-mulched beds have become more problematic because of the extended time between laying the mulch and crop planting.

Weed management challenges also increase for Georgia vegetable growers because they often produce three to five crops on a single installation of plastic mulch spanning 18 to 24 mo before removing the plastic. For each subsequent crop after the initial crop, weeds not only emerge in the row middles but also through holes created in the mulch from previous plantings and natural degradation of the plastic (Boyd 2016). Broadcast-applied herbicide options are limited by the presence of plastic mulch and crop tolerance. Glyphosate and paraquat, two of the most popular options among growers, provide broad-spectrum weed control and can be removed from the plastic mulch before planting with a single rainfall or irrigation event (Boyd 2016; Culpepper et al. 2009; Grey et al. 2009). Many winter annual weeds can be controlled by glyphosate or paraquat; however, wild radish (*Raphanus raphanistrum* L.), cutleaf evening primrose (*Oenothera laciniata* Hill), and horseweed [*Conyza canadensis* (L.) Cronquist] are often present and may not be adequately controlled by these herbicides (Culpepper et al. 2005; Eubank et al. 2008). When glyphosate or paraquat was applied alone, 80% to 81%, 56% to 60%, and 55% to 74% control of wild radish, cutleaf evening primrose, and horseweed was observed, respectively (Culpepper et al. 2005; Eubank et al. 2008). To control these problematic weeds and start the season with minimal weed competition, additional herbicidal tools are needed.

Although numerous herbicides are available to control these weeds, broadcast applications over plastic mulch increase complications. Herbicides applied over plastic mulch wash off of the mulch with an initial irrigation or rainfall event, partially wash off of mulch over time, or bind to the mulch without release (Culpepper et al. 2009; Grey et al. 2009; Grey et al. 2018; Randell et al. 2020). For example, flumioxazin applied over plastic mulch persists, resulting in significant injury and yield reductions for squash and tomato (*Solanum lycopersicum* L.) (Culpepper et al. 2009; Grey et al. 2009). Halosulfuron-methyl applied over plastic mulch, even with large amounts of rainfall after application and before planting, damaged squash, broccoli (*Brassica oleracea* L. var. *botrytis* L.), and cabbage (*B. oleracea* L. var. *capitata* L.) at varying levels (Culpepper et al. 2009; Randell et al. 2020). Thus, identifying potential herbicide tools to improve weed control in vegetables grown on plasticulture must begin with understanding the relationship of the herbicide and the mulch.

Although synthetic auxin herbicides have been available for 70 years, interest in using these chemistries has been renewed, due to formulation improvements along with the introduction of 2,4-D and dicamba-tolerant agronomic crops (Anonymous 2017, 2018a, 2018b; Johnston et al. 2018). The use of these compounds to control many troublesome weeds has been documented for decades. Culpepper et al. (2005) observed that tank mixtures of 2,4-D or dicamba plus glyphosate resulted in 94% to 97% control of both cutleaf evening primrose and wild radish 28 d after treatment. In addition, applications of glyphosate plus either 2,4-D or dicamba resulted in 90% to 99% control of horseweed 4 wk after treatment (Eubank et al. 2008). However, nontolerant broadleaf crops can be extremely sensitive to low doses of these herbicides. Significant injury and yield reductions from low rates of auxinic herbicides have been documented in many high-value vegetable crops, such as bell pepper (*Capsicum annuum* L.), potato (*Solanum tuberosum* L.), snap bean (*Phaseolus vulgaris* L.), squash, and watermelon (Colquhoun et al. 2014; Culpepper et al. 2018; Dittmar et al. 2016).

Although crop response to auxinic herbicides applied at low rates to the foliage of vegetable crops has been documented, more research is needed to investigate the use of preplant applications of 2,4-D or dicamba over plastic mulch before vegetable transplanting. Provided 2,4-D and dicamba can be removed from mulch with rainfall and/or irrigation, or dissipate to nonlethal concentrations rapidly on plastic mulch, the use of these herbicides would provide vegetable growers valuable tools in their weed control program. Therefore, the objective of this experiment was to determine, using analytical and bioassay techniques, the potential for applying 2,4-D or dicamba over plastic mulch before transplanting yellow squash and watermelon.

Materials and Methods

Site Selection and Trial Establishment

Studies were conducted in the summer of 2018 and 2019 near Ty Ty, GA (31.50911°N, 83.64813°W) to evaluate squash and watermelon response to 2,4-D plus glyphosate and dicamba plus glyphosate applied over plastic mulch before transplanting. For this site, the soil was a Tifton loamy sand (fine-loamy, kaolinitic, thermic Plinthic Kandiudults) with 90% sand, 8% silt, 2% clay, 0.64% organic matter, and a pH of 6.3 in 2018; and 84% sand, 12% silt, 4% clay, 0.86% organic matter, and a pH of 6.2 in 2019.

The soil was prepared conventionally and raised beds were formed 3 mo before planting to allow fumigant dissipation before planting. As beds were being formed, the entire trial area was treated with a fumigant system including 1,3-dichloropropene, chloropicrin, and metam sodium. The initial bedder (Hendrix & Dail, Inc., Greenville, NC) formed a prebed 20 cm tall and 81 cm wide while injecting 1,3-dichloropropene and chloropicrin (Pic-Clor 60; TriEst Ag Group, Inc., Greenville, NC) at a rate of 197 L ha^{-1} 20 cm below the bed top using three evenly spaced injection shanks. This was followed immediately by a combination bed shaper and plastic mulch layer (Kennco Manufacturing, Inc., Ruskin, FL) that injected metam sodium (Vapam® HL™; Amvac Chemical Corp., Los Angeles, CA) 10 cm deep with injection shanks 10 cm apart at a rate of 700 L ha^{-1}. As metam sodium was injected, drip tape was placed in the center of the bed 2.5 cm below the bed surface and the entire bed was covered with black on black TIF (Guardian Agro Plastics, Tampa, FL).

The experimental design was a randomized complete block design with an augmented factorial arrangement of treatments consisting of four application timings, three herbicide options, and a nontreated control for comparison. Herbicides included 2,4-D (Embed® Extra; Corteva Agriscience, Indianapolis, IN) at 1,065 and 2,130 g ae ha^{-1} and dicamba (Engenia®; BASF Corp., Research Triangle Park, NC) at 560 g ae ha^{-1}. Glyphosate (Roundup PowerMax II®; Bayer CropScience, St. Louis, MO) at 1,125 g ae ha^{-1} was included with the lower rate of 2,4-D and with dicamba, whereas 2,250 g ha^{-1} was used with the higher rate of 2,4-D. These tank mixtures, formulations, and rates were selected for potential labeling by registrants. Herbicides were applied over plastic mulch at approximately 45, 30, 15, and 1 d before planting (DBP). The 45-DBP applications were made on March 5, 2018, and February 7, 2019; 30-DBP applications were made on March 23, 2018, and February 21, 2019; 15-DBP applications were made on April 4, 2018, and March 7, 2019; and 1-DBP applications were made on April 17, 2018, and March 20, 2019. Rainfall accumulation, solar thermal radiation, and daily maximum and minimum temperature data were collected onsite at a University of Georgia Weather Monitoring Network station (https://weather.uga.edu) and are presented in Table 1.

Herbicide applications were broadcast directly over plastic mulch using a CO_2-pressurized backpack sprayer equipped with AIXR 11002 nozzles for 2,4-D or TTI 110015 nozzles for dicamba (Teejet Technologies, Wheaton, IL). Independent spray systems were used to apply dicamba and 2,4-D treatments, and sprayers were calibrated to deliver 140 L ha^{-1}. Different nozzle types were used for both herbicides because of labeling requirements at the time these studies were conducted. Spray booms were 138 cm long with a nozzle spacing of 46 cm, and booms were held 41 cm above the mulch. Air temperature at the time of application ranged from 4 to 24 C, relative humidity ranged from 39% to 80%, and wind speeds did not exceed 8 km h^{-1}.

Table 1. Environmental data for 2,4-D plus glyphosate and dicamba plus glyphosate removal from totally impermeable film.

Treatment[a]	2018				2019			
	Temperature[b]		Rainfall[c]	Radiation[d]	Temperature		Rainfall	Radiation
	Max C	Min C	cm	MJ m^{-2}	Max C	Min C	cm	MJ m^{-2}
45 DBP	21.3	8.3	11.3	726	20.9	9.9	10.3	516
30 DBP	22.8	9.8	6.3	437	21.4	10.4	9.3	405
15 DBP	22.5	9.4	3.5	235	21.6	9.7	3.7	239
1 DBP	23.5	4.4	0.0	25	17.7	3.8	0.0	22

[a]Abbreviations: DBP, days before planting; Max, maximum; Min, minimum.
[b]Average daily maximum and minimum after application and before planting.
[c]Total rainfall after application and prior to planting.
[d]Sum of total solar radiation after application and before planting.

Analytical Methods

To quantify the removal of 2,4-D, dicamba, and glyphosate from plastic mulch, plastic samples were collected for analysis. Sampling procedures and extraction were based on similar studies (Grey et al. 2009; Grey et al. 2018). Samples were collected approximately 2 h after treatment (0 d after application [DAA]) and at the time of planting (1, 15, 30, and 45 DAA), resulting in a total of two samples treated plot^{-1}. Samples of mulch were collected from each plot, using an open-faced, wooden, square frame with an inside area of 0.1 m^2. A box-cutting knife was used to cut the mulch along the inside edge of the square in preparation for collection. Needle-nose pliers were then used to fold the mulch inward without contacting the treated side, preventing exposure to foreign objects. Samples were stored in plastic bags, frozen upon collection, and stored at −10 C until analysis.

Field-plot replicate-sample integrity was maintained throughout sample collection, preparation, and chemical analysis. For herbicide analysis, samples were removed from the freezer and allowed to come to room temperature before being placed in a 125-mL volumetric flask, which then was sealed with a rubber stopper. Extractions were conducted using 10% methanol with high-performance liquid chromatography water (Fisher Scientific International, Waltham, MA). The extraction volumes were 100 mL. Samples were placed on a reciprocating shaker for 2 h at 200 rpm. Upon removal, sample extracts passed through a 0.2 μM polytetrafloroethylene membrane filter (Fisher Scientific International) that was fitted to a Luer-Lok™ syringe (Fisher Scientific International), and then passed into a 1.5-mL microcentrifuge tube (Fisher Scientific International). Microcentrifuge tubes were sealed and centrifuged at 12,000 rpm for 5 min. A 1,000-μL sample was then transferred into injection vials (Fisher Scientific International). Samples were analyzed with a Waters Acquity Arc Ultra-High Performance Liquid Chromatography system, coupled with a Waters 2998 PDA and Waters QDa Mass Spectrometry Detector (Waters Corporation, Milford, MA). Chromatographic conditions for each herbicide are provided in Table 2 and were adapted from Majzik et al. (2006) for 2,4-D and dicamba. Selectivity was tested by using blank samples with no interfering peaks detected. Calibration curves were constructed using analytical formulations and showed a linear response for all herbicides, with correlation coefficients (R^2) >0.98; residuals were within 20% without the use of internal standards. Recovery samples were prepared in solution on the basis of the respective acid equivalent applied and indicated that recoveries were within acceptable range (96% to 105%).

Crop Establishment and Data Collection

On April 18, 2018, and March 21, 2019, transplant holes were mechanically made in the plastic mulch using a transplant hole punch wheel (Kennco Manufacturing, Inc., Ruskin, FL) in preparation for planting. 'Enterprise' squash (10 cm tall) was transplanted in single rows with a spacing of 1.8 m between beds and 30 cm between plants within a row. Varieties '7197' (2018) and 'Melody' (2019) of seedless watermelons (15 cm tall) were also planted on the same day as hole punching in single rows, with a spacing of 3.7 m between planted beds and 30 cm between plants within a row. Varieties of pollinator diploid watermelons ('8585' in 2018 and 'Premier' in 2019) were planted at the same time as seedless watermelons and were included as every fourth plant in each plot. Plots for each crop were 5.8 m long by 0.6 m wide. Squash and watermelon management including fertility, irrigation, and insect and disease management were conducted in accordance with university recommendations for the region (Kemble et al. 2019).

Visual crop injury (i.e., chlorosis, epinasty, stunting, leaf deformations) was rated on a scale of 0% to 100% (0% being no injury, 100% being crop death) every 7 d beginning 1 wk after planting up to 5 wk after planting. Reductions in squash growth were quantified by measurements across the diameter of the plant, whereas watermelon growth was quantified by measuring the length of the longest vine.

Ten plants plot^{-1} were measured weekly up to 3 wk after planting. Early fresh-weight biomass was obtained by removing six to nine plants at the ground level and collecting weights at 16 to 22 d after planting (DAP). Ten plants remained in each treated plot for harvest data. Squash harvest was initiated on May 14, 2018, and April 22, 2019. Squash were harvested a total of 30 times, 6 d wk^{-1}, with fruit number and weight plot^{-1} recorded for each harvest. Harvests were then split into early harvests (1 to 10) and total harvests (1 to 30) to determine the impacts of treatments on maturity and total yield. Watermelons were harvested once when the nontreated control reached maturity. For each plot, melons were individually counted and weighed on June 27, 2018, and June 18, 2019. Watermelon harvest data was then sorted into three categories: nonmarketable watermelons (<6.8 kg), marketable watermelons (≥6.8 kg), and total watermelon yield to evaluate the impact of treatments on crop maturity. Postharvest biomass was obtained for squash only to assess herbicide damage present at the end of the season.

Statistical Analysis

For analytical data, ANOVA was applied to the data combined across treatment and year to test for interactions. Year-by-treatment interactions were not significant; therefore, data were pooled over year. Years and replications were considered random effects. Regression analysis was performed using SAS nonlinear regression (SAS Institute Inc., Cary, NC) to determine if 2,4-D, dicamba, and

Table 2. Chromatographic analysis settings of methods used to determine herbicide concentrations on plastic mulch.

Herbicide	Column[a]	Mobile phase[b]	Gradient/isocratic				Injection volume	Flow rate	Column temperature	Mode	SIM	Cone Voltage
			Time	%A	%B	%C	µL	mL min^{-1}	C		g mol^{-1}	V
Dicamba	Cortecs C_{18} 4.6 × 50 mm with 2.7 µm of packing	(A) ACN:H_2O (1:9)	0.00	50	40	10	50	1.1	30	ESI Negative	219	15
		(B) ACN:H_2O (9:1)	1.80	60	40	0						
		(C) H_2O	5.40	50	40	10						
2,4-D	Symmetry C_{18} 4.6 × 75 mm with 3.5 µm of packing	(A) ACN:H_2O (1:9)	0.00	50	40	10	50	1.1	30	ESI Negative	219	15
		(B) ACN:H_2O (9:1)	2.69	60	40	0						
		(C) H_2O	8.17	50	40	10						
Glyphosate	Anionic polar pesticide 2.1 × 100 mm with 5 µm of packing	(A) H_2O + 0.9% FA	0.00	10	90	–	10	0.75	40	ESI Negative	168	20
		(B) ACN + 0.9% FA	2.00	10	90	–						
			4.00	60	40	–						

[a]All columns are from Waters Corporation.
[b]Abbreviations: %A (B, C), percentage of treatment A (B, C) in column 3; ACN, acetonitrile; ESI, electron spray ionization; SIM, single ion monitored.

Table 3. First-order dissipation rate constants (k) of 2,4-D, dicamba, and glyphosate from totally impermeable film over time from field experiments conducted in the summer of 2018 and 2019.

Herbicide	Rate	First-order rate constant[a]
	g ae ha^{-1}	d^{-1}
Dicamba	560	0.34 (0.232)[b]a
2,4-D	1,065	0.24 (0.250) a
	2,130	0.17 (0.105) a
Glyphosate	1,125	0.32 (0.103) a
	2,250	0.32 (0.155) a

[a]First-order dissipation rate constants were calculated by nonlinear regression of the herbicide with respect to time (0 to 45 d) using the equation $y = B_0e^{-B1(x)}$. Values for each herbicide and rate for first-order rate constants within a column followed by the same letter are not significantly different at the $P < 0.05$ probability level. General linear model procedures were used with mean separation using 95% asymptotic confidence intervals.
[b]Values represent first-order rate constant (±95% asymptotic confidence interval) and are combined across years.

glyphosate removal from plastic mulch could be described using the exponential decay equation: $y = B_0e^{-B1(x)}$. In this equation, y is the herbicide concentration, B_0 is the initial concentration, B_1 is the slope, and x is sampling time in DAA. After data were regressed against time, the output from the analysis included the first-order dissipation-rate constant (k) (Ohmes et al. 2000). Data for the exponential decay equations were subjected to ANOVA using the nonlinear regression model procedure, with mean separation using 95% asymptotic confidence intervals. Data were then graphed in Sigmaplot 14 (Systat Software, San Jose, CA).

Additional analysis was conducted on herbicide concentration remaining at crop planting with respect to application timing. Because of lack of statistical differences, concentrations remaining at planting were combined across application timings that received rainfall. Concentration means that were present after a rainfall event were analyzed in PROC GLIMMIX in SAS, version 9.4 (SAS Institute Inc.) and means were separated using the Shaffer-simulated method ($\alpha = 0.05$) (Blythe 2012).

Bioassay data were subjected to ANOVA using PROC GLIMMIX in SAS (version 9.4, SAS Institute Inc., Cary, NC 27513) to determine if the combined treatment effects of herbicide, rate, and application timing influenced squash and watermelon growth, development, and yield. The interaction between year and treatment was evaluated and found to be not significant, which allowed us to pool all response variables over year. Rate effects were evaluated for 2,4-D plus glyphosate and were not significant for all response variables; therefore, all 2,4-D data were averaged over rate. Years and replications were considered random effects. All P values for tests of differences between least-squares means were compared and adjusted using the Shaffer-simulated method ($\alpha = 0.05$) (Blythe 2012).

Results and Discussion

Herbicide Removal From Plastic Mulch

Dicamba at 560 g ha^{-1}, 2,4-D at 1,065 and 2,130 g ha^{-1}, and glyphosate at 1,125 and 2,250 g ha^{-1} are theoretically equivalent to 56,000; 106,500; 213,000; 112,500; and 225,000 µg ae m^{-2} applied to plastic mulch, respectively. The exponential decay equation accurately described the removal of all three herbicides from the surface of the mulch with first-order rate constants (k) of 0.34 for dicamba, 0.24 and 0.17 for the 1× and 2× rates of 2,4-D, and 0.32 and 0.32 for the 1× and 2× rates of glyphosate (Table 3; Figures 1 and 2). First-order dissipation rate constants

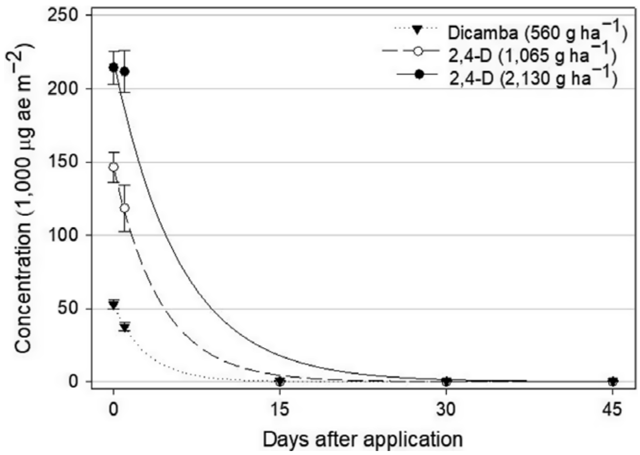

Figure 1. 2,4-D and dicamba removal from the surface of totally impermeable film by rate in Georgia using the exponential decay equation ($y = B_0e^{-B1(x)}$). Nonlinear regression was applied for days after application. The lines represent the first-order regression equation for each treatment. Data points are the means of replications and bars indicate the SE of the mean, as follows: Dicamba 560 g ha^{-1}: $y = 52{,}661.7e^{(-0.339x)}$ ($R^2 = 0.80$; $P < 0.0001$); 2,4-D 1,065 g ha^{-1}: $y = 146{,}866.7e^{(-0.2352x)}$ ($R^2 = 0.71$; $P < 0.0001$); 2,4-D 2,130 g ha^{-1}: $y = 219{,}770.9e^{(-0.1675x)}$ ($R^2 = 0.83$; $P < 0.0001$).

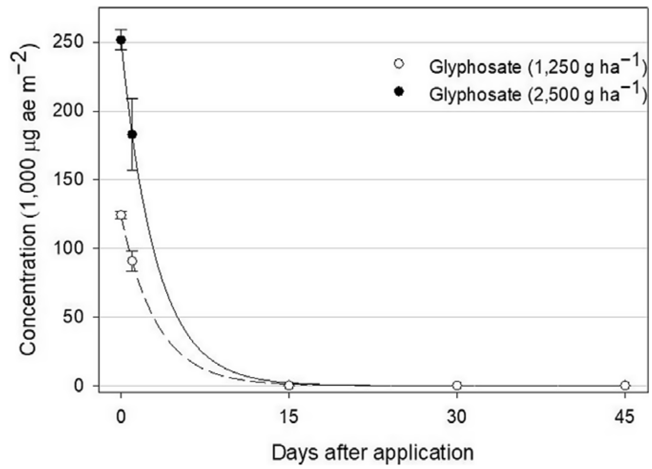

Figure 2. Glyphosate removal from the surface of totally impermeable film by rate in Georgia using the exponential decay equation ($y = B_0e^{-B1(x)}$). Nonlinear regression was applied for days after application. The lines represent the first-order regression equation for each treatment. Data points are the means of replications and bars indicate the SE of the mean, as follows: glyphosate 1,250 g ha^{-1}: $y = 124{,}220.7e^{(-0.3150x)}$ ($R^2 = 0.91$; $P < 0.0001$); glyphosate 2,500 g ha^{-1}: $y = 251{,}723.7e^{(-0.3212x)}$ ($R^2 = 0.91$; $P < 0.0001$).

describing removal over time with respect to all sampling dates were similar for all herbicides (Table 3). For 2,4-D, dicamba, and glyphosate, rainfall was important in herbicide removal from the mulch; 3.5 to 11.3 cm of rainfall accumulated for treatments applied 15 to 45 DBP, whereas no rainfall was observed between application and 1-DBP treatments (Table 3; Figures 1 and 2). These herbicides are not readily photodegraded; however, they have all demonstrated high levels of water solubility (Shaner 2014).

Although removal of 2,4-D and dicamba from the mulch appeared rapid, it is worth noting that when averaged over the 15- to 45-DAA sample dates, there was 31 µg m^{-2} 2,4-D (regardless of rate applied) and 494 µg m^{-2} dicamba remaining on the plastic at planting. For both squash and watermelon, 31 µg m^{-2} is well below the rates of 2,4-D necessary to cause visual injury when applied to the foliage, assuming similar exposure mechanisms (Culpepper et al. 2018; Culpepper and Vance 2019). However, the amount of dicamba remaining on the plastic was enough to cause visual injury on both squash and watermelon when applied to the foliage. Dittmar et al. (2016) reported that when 5 g ha^{-1} or 500 µg m^{-2} dicamba was applied to squash foliage, 50% to 51% visual injury resulted at 10 and 17 DAA. For watermelon, significant vine-length reductions occurred when dicamba was applied to the foliage at as low as a 1/250 field rate, which would be equivalent to 224 µg m^{-2} (Culpepper et al. 2018). Thus, damage should be acceptable from 2,4-D as long as 3.5 to 3.7 cm of rainfall occurs between application and planting and the interval from application to planting is at least 15 d. For dicamba, neither rainfall nor time interval through 45 d will likely alleviate injury concerns.

Glyphosate removal from plastic mulch has been documented (Grey et al. 2009). Previous studies demonstrated that glyphosate can be removed from plastic mulch with as little as a single 1-cm rainfall or irrigation event (Culpepper et al. 2009; Grey et al. 2009). Removal of glyphosate from the surface of plastic mulch in this study followed the same pattern. Averaged over the 15- to 45-DAA application timings with at least 3.5 cm of rainfall, glyphosate at both rates was detected at 118 µg m^{-2}, which is equivalent to approximately a 1/1,000 field rate. Culpepper et al. (2009) demonstrated that tomato and squash could be safely planted after 1 cm of irrigation washed glyphosate from plastic mulch before transplanting. Based on the amount of rainfall received for these studies with plantings of 15 to 45 DAA, glyphosate should not negatively influence crop growth.

Squash Experiments

Pooled over years, visual injury estimates were at their maximum at 23 to 28 DAP. Injury to yellow squash injury influenced by the interaction of application timing and herbicide option ($P < 0.0001$). When herbicides were applied 45, 30, 15, and 1 DBP, squash injury was 12%, 65%, 75%, and 95%, respectively, for dicamba plus glyphosate; and 2%, 4%, 7%, and 73%, respectively, for 2,4-D plus glyphosate (Table 4). Rainfall for each year was similar and the average amounts accumulated after application and before planting were 10.8, 7.8, 3.6, and 0 cm for the aforementioned application intervals, respectively (Table 1). Injury from 2,4-D plus glyphosate was directly related to no rainfall for the 1-DBP application (73%) versus at least 3.6 cm of rainfall for the earlier application timings (2% to 7%). In contrast, dicamba at damaging levels remained on the mulch regardless of rainfall amount or interval between application and planting. Injury was not observed in the control. Although little work has been done using these herbicides preplant in plasticulture production, 2,4-D and dicamba have both been used preplant in row crop production with sensitive crops. When applied up to 4 wk before planting in nontolerant cotton (*Gossypium hirsutum* L.), dicamba caused significant stand loss and leaf distortion, and 2,4-D caused significant stand loss when applied 1 wk before planting (York et al. 2004). In nontolerant soybean [*Glycine max* (L.) Merr.], both 2,4-D and dicamba have the potential to cause significant injury when applied up to 2 wk before planting (Thompson et al. 2007).

Squash canopy widths at 23 to 28 DAP were influenced by the interaction of application timing and herbicide option ($P < 0.0001$). On average, the squash in the control were 78 cm wide (Table 4). Compared with the squash in the control, the 30- (47 cm), 15- (42 cm), and 1- (9 cm) DBP treatments with dicamba plus glyphosate and the 1- (25 cm) DBP treatment with 2,4-D plus glyphosate had significantly smaller width than the

Table 4. Squash injury (23 to 28 DAP), canopy width (23 to 28 DAP), early-season fresh-weight biomass (16 to 22 DAP), and postharvest biomass as influenced by herbicide and application timing.[a]

Herbicide[b]	Application	Injury[c,d]	Width[c,d]	Early biomass[c,d]	Late biomass[c,d]
	DBP	%	cm	g plant^{-1}	
Dicamba plus glyphosate	45	12 (1.2) d	71 (2.6) a	51.0 (4.2) a	3,467 (312) ab
	30	65 (3.1) c	47 (3.2) b	36.6 (4.4) a	2,455 (224) c
	15	75 (2.8) b	42 (2.4) b	36.4 (4.8) a	2,401 (225) c
	1	95 (1.6) a	9 (2.3) d	5.4 (1.2) b	283 (121) d
2,4-D plus glyphosate[e]	45	2 (0.6) e	76 (2.7) a	62.9 (3.8) a	3,834 (176) a
	30	4 (0.8) e	78 (3.6) a	59.0 (4.5) a	3,558 (172) a
	15	7 (1.3) e	74 (2.3) a	52.4 (3.4) a	3,545 (203) a
	1	73 (2.3) b	25 (1.9) c	6.8 (0.9) b	2,712 (136) bc
NTC[a]	NA	0 (0) e	78 (3.3) a	66.2 (3.1) a	3,987 (225) a

[a]Data are pooled over 2018 and 2019.
[b]Abbreviations: DAP, days after planting; DBP, days before planting; NA, not applicable; NTC, nontreated control.
[c]Means followed by the same letter in a column do not differ significantly ($P \leq 0.05$).
[d]Values represent mean (±SE).
[e]2,4-D was applied at 1,065 and 2,130 g ae ha^{-1}; however, results of ANOVA indicated no significant difference between these two treatments for the response variables; therefore, the data were combined for presentation.

Table 5. Yellow squash early yield (harvests 1 to 10) and total yield (harvests 1 to 30) as influenced by herbicide and application timing.[a]

		Harvests 1–10		Harvests 1–30	
Herbicide[b]	Application	No. of fruit[c,d]	Fruit weight[c,d]	No. of fruit[c,d]	Fruit weight[c,d]
	DBP[a]	1,000 ha^{-1}	1,000 kg ha^{-1}	1,000 ha^{-1}	1,000 kg ha^{-1}
Dicamba plus glyphosate	45	171.6 (7.0) a	16.9 (1.2) a	747.6 (14.4) a	80.7 (2.9) a
	30	94.1 (20.3) b	6.9 (1.9) b	558.4 (51.2) b	50.4 (7.2) b
	15	62.8 (11.4) b	3.6 (0.8) b	501.9 (26.9) b	43.2 (3.9) b
	1	0.3 (0.3) d	0.04 (0.04) b	33.1 (17.9) c	2.5 (1.4) c
2,4-D plus glyphosate[e]	45	183.8 (4.0) a	19.1 (1.0) a	829.6 (19.8) a	90.1 (2.5) a
	30	184.4 (5.7) a	18.6 (1.1) a	804.2 (22.2) a	86.0 (3.1) a
	15	178.7 (5.8) a	20.3 (3.1) a	797.9 (20.2) a	88.8 (4.5) a
	1	41.1 (6.3) c	2.2 (0.4) b	555.3 (23.5) b	52.9 (2.7) b
NTC	NA	181.0 (4.2) a	18.9 (0.9) a	828.2 (33.5) a	89.8 (3.5) a

[a]Data were pooled over 2018 and 2019.
[b]Abbreviations: DBP, days before planting; NA, not applicable; NTC, nontreated control.
[c]Means followed by the same letter in a column do not differ significantly ($P \leq 0.05$).
[d]Values represent mean (±SE).
[e]2,4-D was applied at 1,065 and 2,130 g ae ha^{-1}; however, results of ANOVA indicated no significant difference between these two treatments for the response variables, thus, the data were combined for presentation.

control. Early-season biomass at 16 to 22 DAP was influenced by the interaction of application timing and herbicide option ($P < 0.0001$) and followed similar trends to squash widths. On average, early-season squash fresh-weight biomass in the control weighed 66.2 g plant^{-1} (Table 4). Compared to the control, the 30- (36.6 g), 15- (36.4 g), and 1- (5.4 g) DBP treatments with dicamba plus glyphosate and the 1- (6.8 g) DBP treatment with 2,4-D plus glyphosate significantly reduced biomass.

Earliness of harvest is important in vegetable production because it can have a tremendous impact on fruit value (Culpepper et al. 2018). Herbicide injury has the potential to delay maturity in vegetable crops, which can lead to growers receiving less money as they miss their predetermined market window. In an attempt to quantify the potential delay in maturity from treatments, count and weight data from the first 10 harvests were summarized and are reported. Early-harvest fruit counts and weights were significantly affected by the interaction of application timing and herbicide option ($P < 0.0001$). As applications of dicamba mixtures were made closer to planting, early-season fruit counts and weights decreased, whereas for 2,4-D mixtures, yield loss only occurred with the application 1 DBP where rainfall was not received to remove it from the mulch (Table 5). On average, squash from the control yielded 181,000 fruit ha^{-1} weighing 18,900 kg ha^{-1} during the first 10 harvests. For both early-season fruit counts and weight, the 30-, 15-, and 1-DBP treatments with dicamba plus glyphosate and the 1-DBP treatment with 2,4-D plus glyphosate significantly reduced yield 47% to 99% compared to the control.

From the cumulative fruit counts and weights after 30 harvests, a greater yield loss was noted with dicamba applications made closer to planting, whereas 2,4-D mixtures only influenced yield applied 1 DBP. On average, squash in the control yielded 828,200 fruit ha^{-1} weighing 89,800 kg ha^{-1} (Table 5). Season-long fruit count and weight data in response to preplant applications of dicamba or 2,4-D tank-mixtures were nearly identical. For both total fruit counts and weight, the 30-, 15-, and 1-DBP treatments with dicamba plus glyphosate and the 1-DBP treatment with 2,4-D plus glyphosate yielded significantly less than the control.

After the final harvest, squash plants free of fruit were removed at the soil line and weighed to quantify the reduction in nonfruit fresh-weight biomass over the entire season. Postharvest biomass was influenced by the interaction of application timing and herbicide option ($P < 0.0001$). Biomass results followed similar trends to other data. On average, squash plants in the control weighed 3,987 g plant^{-1} (Table 4). Postharvest biomass was significantly

Table 6. Watermelon injury (30 DAP), vine length (23 to 34 DAP), and fresh-weight biomass (16 to 22 DAP) as influenced by herbicide and application timing.[a]

Herbicide[b]	Application	Injury[c,d]	Vine length[c,d]	Biomass[c,d]
	DBP	%	cm	g plant^{-1}
Dicamba plus glyphosate	45	13 (4.8) d	60.8 (2.7) a	21.0 (2.8) a
	30	39 (3.6) c	48.0 (2.2) b	18.0 (1.4) a
	15	42 (5.9) c	48.0 (5.4) b	18.7 (2.4) a
	1	94 (1.7) a	1.5 (1.5) c	1.3 (0.3) b
2,4-D plus glyphosate[e]	45	1 (0.4) e	68.5 (1.6) a	23.0 (2.0) a
	30	2 (0.8) e	66.1 (1.5) a	20.8 (1.4) a
	15	4 (1.4) e	65.2 (3.0) a	17.7 (1.6) a
	1	84 (3.0) b	9.1 (2.8) c	1.6 (0.2) b
NTC	NA	0 (0) e	68.5 (1.8) a	19.5 (2.1) a

[a]Data pooled over 2018 and 2019.
[b]Abbreviations: DAP, days after planting; DBP, days before planting; NA, not applicable; NTC, nontreated control.
[c]Means followed by the same letter in a column do not differ significantly ($P \leq 0.05$).
[d]Values represent mean (±SE).
[e]2,4-D was applied at 1,065 and 2,130 g ae ha^{-1}; however, results of ANOVA indicated no significant difference between these two treatments for the response variables; thus, the data were combined for presentation.

Table 7. Watermelon fruit weight as influenced by herbicide and application timing.[a]

		Watermelon yield[c,d]		
Herbicide[b]	Application	<6.8 kg	≥6.8 kg	Total
	DBP	1,000 kg ha^{-1}		
Dicamba plus glyphosate	45	29.1 (2.8) ab	48.4 (3.6) ab	77.5 (2.7) a
	30	34.9 (6.7) ab	34.3 (6.0) bc	69.2 (2.3) a
	15	33.8 (3.7) ab	33.4 (4.2) bc	67.2 (5.1) a
	1	6.0 (3.7) c	1.8 (1.2) d	7.8 (4.1) c
2,4-D plus glyphosate[e]	45	25.0 (2.0) b	53.1 (2.1) a	78.1 (1.7) a
	30	28.8 (1.7) ab	47.7 (2.8) ab	76.5 (2.2) a
	15	29.9 (2.4) ab	47.8 (2.9) ab	77.7 (2.4) a
	1	23.7 (3.9) b	25.6 (5.2) c	49.3 (6.6) b
NTC	NA	37.9 (3.7) a	42.5 (5.8) ab	80.4 (5.4) a

[a]Data pooled over 2018 and 2019.
[b]Abbreviations: DBP, days before planting; NA, not applicable; NTC, nontreated control.
[c]Means followed by the same letter in a column do not differ significantly ($P \leq 0.05$).
[d]Values represent mean (±SE).
[e]2,4-D was applied at 1,065 and 2,130 g ae ha^{-1}; however, results of ANOVA indicated no significant difference between these two treatments for the response variables; thus, the data were combined for presentation.

reduced by the 30-, 15-, and 1-DBP treatments with dicamba plus glyphosate and the 1-DBP treatment with 2,4-D plus glyphosate compared to the control.

Watermelon Experiments

Visual estimates of watermelon injury were at their maximum 30 DAP in both years and are presented in Table 6. Watermelon injury was influenced by the interaction of application timing and herbicide option ($P < 0.0001$). Dicamba plus glyphosate applied 45, 30, 15, and 1 DBP injured watermelon 13%, 39%, 42%, and 94%, respectively. Sublethal doses of dicamba applied to foliage injure watermelon as well (Culpepper et al. 2018). For 2,4-D plus glyphosate, 84% injury was noted when applied 1 DBP with no rainfall received after application and before planting. Once rainfall of at least 3.5 cm was received to wash the mulch, the subsequent injury was less than 5% when averaged over rate (Table 6).

Watermelon vine-length measurements taken 23 to 34 DAP were significantly influenced by the interaction of application timing and herbicide option ($P < 0.0001$). Watermelon vines in the control averaged 68.5 cm long (Table 6). Only dicamba plus glyphosate applied 30 (48 cm), 15 (48 cm), and 1 (1.5 cm) DBP, and 2,4-D plus glyphosate applied 1 (9.1 cm) DBP significantly reduced vine length. Vine-length reductions of 51% or greater were reported when dicamba was applied to watermelon foliage at a 1/75 rate (Culpepper et al. 2018). Early-season watermelon biomass collected 16 to 22 DAP noted watermelon plants in the control averaged 19.5 g plant^{-1}. Biomass reductions were only noted with dicamba plus glyphosate and 2,4-D plus glyphosate applied 1 DBP (1.3 and 1.6 g plant^{-1}, respectively) (Table 6).

Watermelon fruit counts and weights were analyzed using the aforementioned three categories: nonmarketable watermelons (<6.8 kg), marketable watermelons (≥6.8 kg), and total watermelon yield, to understand treatment effects. Yield data were analyzed and are presented in terms of weight of watermelons picked, because watermelon counts followed similar patterns. Nonmarketable watermelon yield ($P < 0.0001$), marketable watermelon yield ($P < 0.0001$), and total watermelon yield ($P < 0.0001$) were all significantly affected by the interaction of herbicide option and application timing. For nonmarketable watermelon yield, dicamba plus glyphosate applied 1 DBP (6,026 kg ha^{-1}), as well as 2,4-D plus glyphosate applied 45 and 1 DBP (24,967 and 23,708 kg ha^{-1}, respectively) resulted in significant nonmarketable yield loss compared to the control (Table 7). For marketable watermelon yield, both glyphosate plus dicamba or 2,4-D applied 1 DBP (1,837 and 25,590 kg ha^{-1}, respectively) without rainfall to remove any of the herbicide from the mulch caused 40% to 96% yield loss compared to the control. Interestingly, with the glyphosate plus

2,4-D treatment differences observed, the fewer smaller, nonmarketable fruit noted with the 45-DBH application correlated with a higher number of marketable fruit, whereas the 1-DBH application had not only fewer small fruit but also fewer marketable and total fruit when compared to the control. Marketable watermelon yield reductions when exposed to a 1/75 rate of either 2,4-D or dicamba early in the season have been reported (Culpepper et al. 2018). Total watermelon yield followed the same trend as marketable watermelon yield, with only dicamba and 2,4-D applied 1 DBP (7,863 and 49,298 kg ha^{-1}, respectively) significantly reducing yield compared to the control.

Dicamba and 2,4-D would be useful in helping vegetable producers control problematic weeds before planting in a multicrop vegetable plasticulture system. Although dicamba removal from plastic mulch seemed rapid with respect to time in the analytical analysis, the amount of dicamba remaining on the mulch caused significant damage to both squash and watermelon. Generally, the greater the time between application and planting, the more crop tolerance was observed, but excessive injury and growth reductions from dicamba on the plastic mulch prohibit potential labeling for the herbicide in plasticulture production systems. In contrast, 2,4-D demonstrated a significant potential for use in a plasticulture weed management system for vegetable growers. Minimal visual injury, growth reductions, and yield loss were observed for both squash and watermelon as long as rainfall of at least 3.5 cm occurred after application and before crop planting. Furthermore, we found no differences between 2,4-D mixtures applied at a 1× or 2× rate, with safety observed with applications when rainfall was received before planting. However, weed management in multicropped plasticulture vegetable production is complex. Future research should be conducted to evaluate the use of 2,4-D in a multicropped plasticulture system, where holes are present in the mulch, evaluating potential residual uptake of 2,4-D. Also, other valuable crops produced in plasticulture systems such as tomatoes, bell peppers, or cole crops should be evaluated.

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