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## **Research Article**

**Cite this article:** Kalina JR, Corkern CB, Shilling DG, Basinger NT, Grey TL (2022) Influence of time of day on dicamba and glyphosate efficacy. Weed Technol. **36**: 21–27. doi: 10.1017/wet.2021.66

Received: 12 August 2020 Revised: 7 January 2021 Accepted: 9 August 2021 First published online: 13 August 2021

**Associate Editor:** William Johnson, Purdue University

#### Nomenclature:

Auxin; dicamba; glyphosate; pitted morningglory; *Ipomoea lacunosa* L.; prickly sida; *Sida spinosa* L.; sicklepod; *Senna obtusifolia* L.; cotton; *Gossypium hirsutum* L.

#### **Keywords:**

Herbicide-resistant crops; auxinic herbicide; genetically engineered; circadian rhythm

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# Influence of time of day on dicamba and glyphosate efficacy

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## Abstract

Renewed interest in studying auxin herbicides (WSSA Group 4) is increasing as a result of the release of genetically engineered crop varieties that are tolerant to preemergence and postemergence applications of specific formulations of dicamba. Auxin-resistant crops were developed in response to the development of weed species resistant to glyphosate and other herbicides. Research was conducted at multiple field locations in Georgia in 2018 and 2019 to examine weed control when postemergence herbicides were applied to dicamba- and glyphosate-resistant cotton at eight different points in time over a 24-h period. Applications were made at 1 h prior to sunrise all the way up to midnight during the same day to examine the effect of herbicide application timing on broadleaf weed control. Glyphosate, dicamba, and glyphosate plus dicamba were applied at each timing. Visual ratings of weed control were scored at 7, 14, 21, and 28 d after treatment (DAT). Weed control was affected by herbicide application timing. Midnight applications resulted in the lowest levels of control. Sicklepod, pitted morningglory, and prickly sida control was 49%, 38%, and 41%, respectively. Greatest control of all three species (up to 99%) occurred from the noon to 1 h prior to sunset application timings. Orthogonal contrasts of timing of application indicated that weed control was improved with day > night and pre-dawn > midnight.

#### Introduction

Dicamba is a selective herbicide that is most effective on broadleaf weed species with little to no response on grasses (Chang and Vanden Born 1971). It belongs to the synthetic auxin (WSSA Group 4) family of herbicides (Shaner et al. 2014) and has weed control utility across production systems. Auxin herbicides have been registered for broadleaf weed management in wheat (Triticum aestivum L.), rice (Oryza sativa L.), and maize (Zea mays L.) since their inception in the 1940s, and are also used in turf, pasture, and residential areas. In 2017, Monsanto Company (now Bayer Crop Sciences) released genetically engineered crop systems for cotton (Gossypium hirsutum L.) and soybean [Glycine max (L.) Merr.] that are resistant to dicamba under the trade name Xtend<sup>™</sup> but restricts the use to dicamba formulations that are designed to reduce off-target movement (Anonymous 2020). Whereas glyphosate-resistant crops included gene insertion to change the pathway of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), creating a glyphosate-resistant form of the EPSPS enzyme (Dill 2005), auxinic-resistant crops rely on the plant's ability to metabolize the applied herbicide (Green 2012). The need for new crop technologies was prompted by the development of resistance in numerous weed species such as Palmer amaranth (Amaranthus palmeri S. Watson) to multiple herbicides (Heap 2018). These modes of action (MoAs) included acetolactate synthase (ALS) inhibitors (WSSA Group 2) (Tranel and Wright 2002), triazines (WSSA Group 5) (Darmency and Gasquez 1990), glyphosate (WSSA Group 9) (Culpepper et al. 2006), and several others (Heap 2018).

Synthetic auxin herbicides are structurally similar to endogenous indole-3-acetic acid (IAA) and are therefore capable of following the IAA pathway in plants (Grossman 2000, 2010). Dicamba and other auxin analog herbicides are highly symplastically mobile in plant tissue, accumulating in actively growing tissue (Chang and Vanden Born 1971). Most translocation occurs within 12 h of application (Johnston et al. 2018). The initial symptomology appears as stem elongation (epinasty) and leaf cupping, followed by chlorosis and necrosis, with plant death occurring between 10 and 18 d after treatment (DAT) (Sciumbato et al. 2004). Dicamba and other synthetic auxin herbicides bind to the TIR1 receptor, causing degradation of the Aux/IAA repressor, leading to the constant transcription of auxin response factors (Grossman 2010). Overproduction of ethylene is one of the results of deregulation in these response factors and

generally leads to rapid epinasty in dicots (Grossmann 2000). This overproduction of ethylene leads to accumulation of high amounts of abscisic acid that remain in the shoot tissue; this is thought to be the main mechanism of plant death in dicots (Scheltrup and Grossman 1995).

The widespread adoption of glyphosate-resistant crops (Young 2006) led to glyphosate being the most widely used pesticide in the United States (USDA 2020). This widespread use in agronomic crop production has led to the selection of herbicide-resistant biotypes of several weed species. Glyphosate is a WSSA Group 9 herbicide that has broad-spectrum activity on both monocots and dicots (Shaner et al. 2014). Growers often prefer to apply glyphosate as a tank mixture with other herbicides, and the addition of glyphosate may help growers control other weed species against which dicamba alone would be weak (Underwood et al. 2017). The MoA of glyphosate works by inhibiting the biosynthesis of aromatic amino acids through the EPSPS pathway (Amrhein et al. 1980). This inhibits the production of many necessary amino acids and plant proteins, causing relatively slow (7 to 21 d) plant death (Sprankle et al. 1975). Fifty-three weed species have been confirmed resistant to glyphosate (Heap 2021). Despite the herbicide resistance issues that have developed, glyphosate remains a highly valuable herbicide in crop production because of its continued activity on many weed species (Beckie et al. 2020).

Herbicides affect normal plant processes, and their activity can be influenced by time of day (TOD) because of factors such as light intensity, temperature, and other environmental factors and stressors (Stopps et al. 2013; Walker and Oliver 2008). Previous studies have reported variability in weed control based on the TOD of herbicide application (Norsworthy et al. 1999; Stopps et al. 2013). Herbicides that lead to plant death by inhibiting the light reactions of photosynthesis would be affected by the TOD of application and thus light/dark conditions, as their MoA specifically targets reactions involved in photosynthesis (Bowes et al. 1980; Matringe et al. 1992; Shaner et al. 2014). Other MoAs that do not directly affect photosynthetic activity have also been observed to be influenced by the TOD application (Duke and Kenyon 1986; Mohr et al. 2007; Norsworthy et al. 1999). The reason for these observed differences can be explained by the differential translocation that plants exhibit based on diurnal cycles (Johnston et al. 2018). Light can influence the translocation of photosynthates based on the storage and mobilization of sugars produced during photosynthesis, which would suggest that these herbicides would move in a similar fashion dependent on their physiochemistry (Lanoue et al. 2018). Translocation is key to systemic herbicide efficacy, because significant movement within the plant is imperative to reach the site of action (Walker and Oliver 2008).

In addition to differential translocation based on photosynthetic activity, plant morphology can also change with diurnal patterns (Hammerton 1967). Diurnal leaf movement has been shown to affect spray droplet interception in some species such as sicklepod, and consequently would reduce herbicide absorption and efficacy (Norsworthy et al. 1999). Furthermore, the TOD effect has been seen across several cropping systems, multiple MoAs indicating that most applications occurring during high-light midday applications tended to result in greater herbicide efficacy (Budd et al. 2017; Carter and Prostko 2019; Mohr et al. 2007; Montgomery et al. 2017; Stewart et al. 2009; Stopps et al. 2013). Previous studies have indicated that additions of glyphosate to dicamba applications can improve control of glyphosate-resistant waterhemp (*Amaranthus rudis* J.D. Sauer); this efficacy could be translatable to Palmer amaranth. However, the TOD effect of glyphosate, dicamba, and the combination with glyphosate has not been investigated in cotton or other dicamba-tolerant crops. Therefore, the objectives of this study are to determine the overall weed control efficacy of dicamba and glyphosate in cotton varieties that are tolerant to these herbicides. The focus of the study was on the time of application, within a 24-h period, using dicamba, glyphosate, and a mixture of both herbicides, and to determine the effect of weed height and TOD on herbicide efficacy. Although this study focuses on dicamba, glyphosate was included because it is affected by photoperiod differences (McAllister and Haderlie 1985), as well as being a common mixture with dicamba in the Georgia cotton-growing region (Underwood et al. 2017). Experiments evaluated the influence on weed control of diurnal time of herbicide application of dicamba, glyphosate, and a combination of the two.

## **Materials and Methods**

Field studies were conducted in 2018 and 2019 at Tift (31.39° N, 83.55° W) and Sumter (32.03° N, 84.37° W) counties in Georgia. Four species of weeds were evaluated: sicklepod, prickly sida, pitted morningglory, and ivyleaf morningglory (Ipomoea hederacea L.). These species were chosen because they are problematic in cotton production (Van Wychen 2016). Immediately before cotton planting, weed seed (Azlin Seed Service, Leland, MS) was hand-sown over the entire area with a hand-operated broadcast spreader (EarthWay Commercial Spreaders Inc., Bristol, IN) and incorporated through the bedding and planting process. The weed seed was mixed uniformly by volume before sowing and applied to the trial area at approximately 7 L ha<sup>-1</sup>, resulting in a stand of 15 to 20 plants  $m^{-2}$  of each species at the time of application. The cotton cultivar, 'Deltapine 1646 B2XF' (Bayer Crop Sciences, St. Louis, MO), was planted with a row spacing of 0.9 m and a population of 5 to 7 plants m<sup>2-</sup>. Cotton planting dates ranged between early and late May dependent on the availability of equipment and environmental conditions. Herbicide was applied 4 to 7 wk after planting relative to weed maturity and cotton development.

In Tift County, the soil type for both test areas was a Tifton sandy loam (96% sand, 2% silt, 2% clay, 1.2% organic matter, and pH 6.0 to 6.5, fine-loamy, kaolinitic, thermic Plinthic Kandiudults), whereas Sumter County soil was Greenville sandy loam (71% sand, 13% silt, 16% clay, 1% organic matter, and pH 6.0 to 6.5, fine-loamy, kaolinitic, thermic Rhodic Kandiudults). All agronomic factors relating to irrigation, fertility, and pest control for insects and fungi, were based on The University of Georgia Extension recommendations for Georgia cotton production (Whitaker 2019).

Plots were arranged in a randomized complete block utilizing a 3 by 8 by 2 factorial, with four replications. Plot size was two rows of cotton 9.1 m long by 0.9 m wide (typical row spacing for southern Georgia). The randomized factors were time (8), herbicide treatment (3), and weed size (6 to 10 and 11 to 18 cm) (2). Visual ratings were taken at 7, 14, 21, and 28 DAT on a scale of 0 (no control) to 100% (total death). Following the last visual rating, plots at each location were maintained weed-free until harvest, using mechanical and chemical means following Georgia pesticide recommendations and herbicide labels (Whitaker 2019). Within the study, herbicides included glyphosate (1.3 kg ae ha<sup>-1</sup>), dicamba (0.56 kg ae ha<sup>-1</sup>), or a mixture of glyphosate plus dicamba at the same use rates [Xtendimax and Roundup Powermax II, respectively (Monsanto Company, St. Louis MO)]. These three herbicide treatments were applied at eight different times within a 24-h period (Table 1). Two different treatment scenarios were utilized

Table 1. Solar radiation (PPF) at herbicide application timings.<sup>a,b</sup>

Timing	PPF	
5:30 AM	1 h prior to sunrise	0 e
7:30 AM	1 h after sunrise	1,050 c
9:00 AM	Midmorning	1,580 b
12:00 PM	Noon	1,840 at
3:00 PM	Mid-afternoon	1,940 a
7:30 PM	1 h prior to sunset	450 d
9:30 PM	1 h after sunset	0 e
12:00 AM	Midnight	0 e

<sup>a</sup>Averaged across locations because of lack of variance (ANOVA PROC GLM).

<sup>b</sup>PPF, photosynthetically active light (µmol m<sup>-2</sup> s<sup>-1</sup>) readings were obtained from the University of Georgia Weather Network for Sumter and Tift counties Georgia weather stations (http://georgiaweather.net/). Solar radiation measurements were recorded to ensure that treatments were applied at uniform conditions and means separated using Tukey-Kramer HSD  $\alpha = 0.05$ . Levels not connected by the same letter are significantly different.

with a 10- to 14-d separation to achieve different weed sizes (6 to 10 cm and 11 to 18 cm tall). The eight different applications were chosen to represent times of the day that a grower may be applying herbicide and different amounts of solar radiation when the herbicide is applied based on the TOD (Table 1). Solar radiation measurements were also taken at the time of each herbicide application (Table 1) to ensure that applications were made in uniform conditions concerning light intensity. Data on solar radiation were averaged across year and location (ANOVA P = 0.2379).

Applications were made using a 91.4-cm  $CO_2$ -pressurized handheld boom with three nozzles spaced at 47.5 cm calibrated to deliver 140 L ha<sup>-1</sup>. TeeJet TTI 11002 nozzles (TeeJet<sup>®</sup> Technologies, Springfield, IL) were used to maintain consistency with label requirements for applying XtendiMax<sup>®</sup> herbicide. Treatments including XtendiMax also contained Reign driftreducing agent (Loveland Products, Greenville, MS) at 0.5% vol/ vol to follow label recommendations.

Data taken on visual ratings of overall weed control by species were subjected to an ANOVA using JMP v14.3.1 with a significance value of  $\alpha = 0.05$ . Tukey-Kramer HSD means separation was utilized to reduce the risk of type I errors (Abdi and Williams 2010). Data were combined over location because of lack of variance (ANOVA P < 0.001) for each species observed at both location.

Orthogonal contrasts were used to compare data for weed control (SAS version 9.4; SAS Institute, Cary, NC) across pre-dawn and midnight applications to determine the possible influence of circadian rhythm on the efficacy of these herbicides. Comparisons were also made to determine control between daylight and night applications. Pre-dawn (5:30 AM) was compared against midnight applications, and light (7:30 AM to 7:30 PM) applications were compared against all dark (9:30 PM to 5:30 AM) ratings 21 DAT. Data reported are the visual ratings made at 21 DAT because of the presence of the full herbicide effect as well as reduced risk of the presence of newly emerged weeds.

Once cotton reached 90% to 95% open bolls, it was defoliated following The University of Georgia Extension recommendations for Georgia cotton production (Whitaker 2019). Cotton was harvested and weighed by plot for all locations. Tift County cotton was not collected in 2018 because of inclement weather (Hurricane Michael). All seed cotton weights were measured at the time of harvest. Yield data were subjected to ANOVA in JMP v14.3 to analyze variance between TOD and herbicide treatment. Data were combined across year in Sumter County and location because of lack of variance (P = 0.4394 and 0.0953, respectively). Means were separated using Tukey-Kramer HSD ( $\alpha = 0.05$ ). Orthogonal contrasts (SAS 9.4) were used to compare herbicide treatments to the non-treated control.

#### **Results and Discussion**

Results of the ANOVA at 21 DAT (P values > 0.05 for location and experiment year) led to weed control data being combined over year and location. The ratings at 21 DAT were chosen to represent when herbicides had a full effect on control, and new weed growth was not yet observed, as no preemergent herbicides were used at planting. Interactions observed from the ANOVA included time of application (P < 0.0001), weed species (P = 0.0069), and size (P < 0.0001), as well as the herbicide (P < 0.0001) that was applied. Results are reported by species (P < 0.0001), as they all responded differently.

### Sicklepod

Sicklepod responded to glyphosate applications most significantly between day and night timings using Tukey-Kramer HSD ( $\alpha = 0.05$ ) for means comparison (Table 2). Diurnal nyctinastic leaf movement during hours of darkness probably resulted in less interception of the applied herbicide (Kraatz and Anderson 1980), as has been noted by others (Dalazen and Merotto 2016; Norsworthy et al. 1999). Dicamba application showed a smaller effect based on TOD. However, the mixture of dicamba and glyphosate did show differences from TOD for both weed heights. The lowest overall efficacy observed for sicklepod occurred during midnight applications, where glyphosate was able to achieve only 49% control. Noon applications resulted in up to 99% control using the mixture (Table 2).

#### Morningglory Species

Visual ratings of control for morningglory spp. represented both Ipomoea hederacea and I. lacunosa. Levels of control using dicamba only showed differences relative to the TOD at the larger (11 to 18 cm) weed size (Table 3). Overall control of the larger weeds increased at midday, suggesting that although both herbicides are phloem-mobile, dicamba translocates differently than glyphosate does. TOD effect on glyphosate activity was similar to dicamba, but greater differences were observed at the smaller (6 to 10 cm) weed size. Interestingly, although total weed control was increased with the mixture (P < 0.0001, data not shown), effects were greater concerning TOD--possibly a result of the two herbicides' interaction with each other in both the spray tank and the plant tissue (Underwood et al. 2017). Morningglory control increased by nearly 50% total control in most cases when treatments were applied at midday and early afternoon and were lower when applied during dark conditions. Similar to sicklepod control, morningglory spp. showed the least herbicide response during midnight glyphosate applications at 38%, whereas the mixture applied at noon resulted in up to 99% control (Table 3). As dicamba is required in lower use rates, to affect weeds the area of a leaf that must be covered by herbicide solution is less; thus, in the case of a large-leaf weed like morningglory, dicamba can maximize its efficacy (Bauerle et al. 2015).

#### Prickly Sida

The TOD effect was observed for prickly sida at both heights (6 to 10 cm and 11 to 18 cm) but was more pronounced at midnight with

Table 2. Time-of-day control response of sicklepod in Tift and Sumter counties, Georgia, 21 d after treatment. Means were combined between 2018 and 2019 and location because of ANOVA results (P < 0.005).

		Dicamba		Glyphosate		Dicamba plus glyphosate	
Timing		6-10	11-18	6-10	11-18	6-10	11–18
				Control %ª			
5:30 AM	Pre-dawn	85 a	76 bc	61 c	69 b	89 abc	88 ab
7:30 AM	Day	92 a	85 ab	83 abc	89 a	95 ab	95 ab
9:00 AM	Day	91 a	89 ab	81 abc	89 a	98 a	93 ab
12:00 PM	Day	88 a	88 ab	92 a	90 a	99 a	97 a
3:00 PM	Day	80 a	89 ab	89 ab	91 a	98 a	97 ab
7:30 PM	Day	86 a	92 a	86 ab	86 ab	97 a	96 ab
9:30 PM	Night	81 a	76 abc	79 abc	64 bc	84 bc	81 bc
12:00 AM	Night	80 a	68 c	68 bc	49 c	84 c	72 c
	-		Orthogonal contrasts <sup>b</sup>				
	Dicamba		Day > Night	-	NS <sup>c</sup>		
			P < 0.0001		P = 0.1523		
	Glyphosate		Day > Night		NS <sup>c</sup> p=0.4282		
			P < 0.0001				
	Dicamba plus glyphosate		Day > Night		Pre-dawn > Midnight		
			P < 0.0001		P < 0.0001		

<sup>a</sup>Means separated using Tukey-Kramer HSD ( $\alpha = 0.05$ ). Levels not connected by the same letter are significantly different with respect to column.

<sup>b</sup>Day is considered any treatments made 1 h after sunrise (7:30 AM) to 1 h pre-sunset (7:30 PM)--night being all other treatments. Pre-dawn is considered as 1 h prior to sunrise (5:30 AM). <sup>c</sup>No significant difference was observed at α = 0.05.

**Table 3.** Time-of-day control response of morningglory (*Ipomoea* spp.) in Tift and Sumter counties, GA, 21 d after treatment. Means were combined between 2018 and 2019 and location because of ANOVA results (P < 0.05).

		Dicamba Glyphosate		Dicamba plus glyphosate							
			Height at time of herbicide application								
					cm						
Timing		6-10	11-18	6-10	11-18	6-10	11-18				
					Control						
					% <sup>a</sup>						
5:30 AM	Pre-dawn	87 a	76 abc	60 b	61 ab	79 b	83 bcd				
7:30 AM	Day	89 a	78 abc	68 ab	72 a	93 a	88 abc				
9:00 AM	Day	87 a	88 a	76 ab	77 a	97 a	90 abc				
12:00 PM	Day	91 a	85 ab	88 a	76 a	99 a	96 a				
3:00 PM	Day	83 a	85 ab	80 ab	78 a	98 a	93 ab				
7:30 PM	Day	83 a	88 a	63 b	65 ab	91 a	92 ab				
9:30 PM	Night	84 a	71 bc	69 ab	47 bc	88 ab	80 cd				
12:00 AM	Night	81 a	67 c	65 ab	38 c	77 b	73 d				
	Orthogonal contrasts <sup>b</sup>										
	Dicamba		Day > night		Pre-dawn > midnight						
			P < 0.0001		P = 0.0022						
	Glyphosate		Day > night		Pre-dawn > midnight						
			P < 0.0001		P = 0.0014						
	Dicamba plus glyphosate		Day > night		Pre-dawn > midnight						
			P < 0.0001		P = 0.0004						

<sup>a</sup>Means separated using Tukey-Kramer HSD  $\alpha$ =0.05. Levels not connected by the same letter are significantly different with respect to column.

<sup>b</sup>Day is considered any treatments made 1 h after sunrise (7:30 AM) to 1 h before sunset (7:30 PM)---night being all other treatments. Pre-dawn is considered to be 1 h prior to sunrise (5:30 AM).

the larger (11 to 18 cm) weeds (Table 4). Previous studies using different herbicides noted that prickly sida may be less susceptible to the TOD effect (Stewart et al. 2009). The response of prickly sida to glyphosate did, however, follow a pattern similar to the other weed species, with the midday application generally being the most efficacious. A similar trend was seen with the mixture of both

herbicides. Overall control of prickly sida was lowest (P < 0.0001, data not shown) when compared to other weeds in the study, but the mixture did show an overall increase in efficacy. Mixing glyphosate and dicamba increased overall efficacy but followed similar trends; glyphosate at midnight resulted in only 41% control, and the mixture at 3:00 PM resulted in 98% control.

Table 4. Time-of-day control response of prickly sida in Tift and Sumter counties, GA, 21 d after treatment. Means were combined between 2018 and 2019 and location because of ANOVA results (P < 0.005).

Dica		Dicamba		Glyphosate		Dicamba plus glyphosate	
Timing		6-10	11-18	6-10	11-18	6-10	11-18
		control %ª					
5:30 AM	Pre-dawn	70 a	61ab	52 b	46 b	69 cd	74 ab
7:30 AM	Day	71 a	69 a	60 ab	74 a	79 bc	79 a
9:00 AM	Day	73 a	78 a	73 ab	78 a	91 ab	83 a
12:00 PM	Day	75 a	78 a	83 ab	83 a	96 a	89 a
3:00 PM	Day	70 a	86 a	92 a	86 a	95 a	98 a
7:30 PM	Day	76 a	84 a	72 ab	79 a	89 ab	90 a
9:30 PM	Night	64 a	60 ab	58 ab	38 b	59 d	82 ab
12:00 AM	Night	72 a	46 b	55 b	41 b	65 cd	55 b
	Orthogonal contrasts <sup>b</sup>						
	Dicamba		Day > night		NS <sup>c</sup>		
			P < 0.0001		P = 0.0736		
	Glyphosate		Day > night		NS <sup>c</sup>		
			P < 0.0001		P = 0.7369		
	Dicamba plus Glyphosate		Day > night		Pre-dawn > midnight		
			P < 0.0001		P = 0.0030		

<sup>a</sup>Means separated using Tukey-Kramer HSD α=0.05. Levels not connected by the same letter are significantly different with respect to column.

<sup>b</sup>Day is considered any treatments made 1 h after sunrise (7:30 AM) to 1 h before sunset (7:30 PM)--night being all other treatments. Pre-dawn is considered to be 1 h prior to sunrise (5:30 AM). <sup>c</sup>No significant difference was observed at α = 0.05.

Table 5.	Seed	cotton	yield for	Tift	County	(2019)	and	Sumter	County,	GA,	for
2018 and	2019	. Means	combine	d ov	ver year	and lo	catio	n.			

Timing		Nontreated control <sup>a</sup>	Dicamba	Glyphosate	Dicamba plus glyphosate				
				kg ha⁻¹					
5:30	AM	1,793 a	2,311 a	2,154 a	2,527 a				
7:30	AM	1,793 a	2,464 a	2,262 a	2,595 a				
9:00	AM	1,793 a	2,359 a	2,187 a	2,652 a				
12:00	PM	1,793 a	2,522 a	2,310 a	2,727 a				
3:00	PM	1,793 a	2,499 a	2,203 a	2,564 a				
7:30	PM	1,793 a	2,386 a	2,151 a	2,659 a				
9:30	РМ	1,793 a	2,483 a	2,216 a	2,508 a				
12:00	AM	1,793 a	2,268 a	2,096 a	2,554 a				
Orthogonal contrast <sup>b</sup>									
Time of	day	Herbicide > No	herbicide	Р	< 0.0001				

<sup>a</sup>Nontreated control was used as a comparison for yield loss.

<sup>b</sup>Means separated using Tukey-Kramer HSD  $\alpha$ =0.05. Levels not connected by the same letter are significantly different with respect to column.

The use of orthogonal contrasts supports a circadian-rhythm effect concerning the efficacy of some herbicides on some weed species. The pre-dawn applications showed greater efficacy than the midnight applications for the mixture of dicamba and glyphosate in all species (Tables 2, 3, 4). For morningglory, the circadian-rhythm effect was observed across all three herbicidal treatments (Table 3).

An explanation for this observed effect across all three weed species and the herbicides applied would be the difference in the way plants mobilize photosynthate between starch and simple sugars (Geiger et al. 2000). Both herbicides translocate within plant tissue (Wyrill and Burnside 1976; Chang and Vanden Born 1971) and would likely follow the route of the mobilization of photosynthate (Geiger et al. 1986). The activity of photosynthesis based on light intensity has also been shown to drive herbicide efficacy (Kudsk and Kristensen 1992) as well as photosynthate production (Peeters and Eilers 1978). As data on solar radiation (Table 1) show, light intensity being significantly higher in the hour after sunrise than the hour before sunset could explain the differential in the overall efficacy of these herbicide treatments, most likely resulting from several physiological processes (Tables 2, 3, 4).

Yield data showed no differences between application TODs (Table 5)—a trend observed in peanut (*Arachis hypogaea* L.), soybean, and corn (Carter and Prostko 2019; Mohr et al. 2007; Stewart et al. 2009; Stopps et al. 2013). The explanation for this finding is likely that although weed control was reduced during night applications, this reduction was insufficient to affect yield. Although plots were maintained weed-free following the final rating date, any of the infestation following treatment would fall in the critical period of weed control for cotton (Webster et al. 2009). Although lower weed control is never desired, it still can aid in cotton's ability to compete with weed pressure and increase yield at the end of the year compared to no weed control at all (Buchanan and Burns 1970).

The diurnal effects of herbicide efficacy in this study suggest that midday applications of these herbicides will result in the greatest efficacy. Other studies have noted a similar trend, where applications occurring during midday result in greater weed control (Budd et al. 2017; Carter and Prostko 2019; Mohr et al. 2007; Montgomery et al. 2017; Stopps et al. 2013). These studies included several herbicides across MoAs but tended to have increased efficacy when applied under the high-light conditions at midday. It is important to note that the light-adapted treatments show greater efficacy when comparing through orthogonal contrast. However, timely weed control is a major factor, especially when applying dicamba. Control was reduced with increasing weed height when using only dicamba, but overall control improved when glyphosate

was added to control glyphosate-resistant waterhemp (Spaunhorst and Bradley 2013).

Moving forward using this new technology, we must understand how best to maximize efficacy while minimizing off-target impacts. Several of these application timings are outside of label restrictions for dicamba because of off-target movement concerns, but these times of application did result in the lowest average weed control across all species. When balancing efficacy, critical weed size, and unintended off-target impacts, flexibility in the time of application can be a useful tool for growers. As fewer producers are growing on more hectarage, timing of applications becomes more difficult. Understanding the implications of TOD of application and weed size of these herbicides is critical for maximizing efficacy and minimizing the development of herbicide resistance, and may help growers prioritize field operations. With the adoption of dicamba technology, understanding factors including TOD is critical to stewarding this herbicide. As part of this new technology, it is critical to consider all weed control options as part of an integrated weed management strategy. Nonchemical, biological, and mechanical weed control measures must be implemented as part of an overall weed management plan to ensure maximum weed control. Future work could evaluate the impact of these integrated weed management techniques such as cover crops, and the impact of herbicide tank mixes to mitigate TOD effects.

**Acknowledgments.** The authors would like to thank the University of Georgia, Bayer Crop Science, and the Georgia Commodity Commission for Cotton for supporting this research. No conflicts of interest have been declared.

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