

## Confirmation of dicamba-resistant Palmer amaranth in Tennessee

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## Research Article

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**Abstract**

Palmer amaranth has a long history of evolving resistance to herbicides to the point at which it has become a significant obstacle to row crop production. A survey of Palmer amaranth escapes in dicamba-resistant cotton and soybean fields in Tennessee was conducted in fall 2021 with the objective of determining whether poor control was due to environmental phenomena or the development of dicamba resistance. A greenhouse dicamba dose-response screen was conducted on 15 Tennessee accessions. Three accessions were identified with a relative resistance factor between 1.85 and 2.49, and one accession from Lauderdale County was found with a relative resistance factor of 14.25. The Lauderdale County 1 accession developed a higher dicamba resistance level than all others evaluated and can no longer be effectively controlled using dicamba. The history of Palmer amaranth escaping dicamba in the Lauderdale County 1 location from 2019 to 2021 in the field and in preliminary greenhouse screens would suggest that the dicamba resistance has passed between generations. This research documents the first findings of Palmer amaranth control failures in cotton and soybean fields due to the evolution of dicamba resistance.

**Introduction**

From 2012 to 2022, U.S. growers planted more than 30 million hectares of soybean and 4 million hectares of cotton each year (USDA-NASS 2022). Weeds are the largest threat to United States soybean and cotton production, with the potential to decrease yields by  $\geq 36\%$  if left uncontrolled (Oerke 2006). In 2016, new transgenic cultivars became commercially available for soybean and cotton producers, with resistance to 2,4-D or dicamba, in addition to glufosinate and glyphosate, thereby increasing the number of over-the-top herbicide options for growers. Corn and soybean plants with resistance to 2,4-D- and dicamba were developed through the insertion of the *AAD-1* (aryloxyalkanoate dioxygenase-1) transgene and *dicamba monooxygenase* gene, respectively, resulting in herbicide detoxification (Behrens et al. 2007; Braxton et al. 2017; Inman et al. 2016). The year after commercialization, herbicide formulations of 2,4-D and dicamba with low volatility received approval from the Environmental Protection Agency for use in these new soybean and cotton weed management systems. These two synthetic auxinic herbicides selectively control broadleaf weeds such as Palmer amaranth, and when applied in a timely manner, are effective at controlling weeds after they emerge (Cahoon et al. 2015; Manuchehri et al. 2017).

Before 2017, total dicamba use in the United States was estimated at less than 6 million kg per year (USGS 2021). Since the commercialization of dicamba-resistant crops and subsequent labeling of the herbicide for in-crop use, more than 15 million kg of dicamba is now applied across the United States; 10 of those 15 million kg were used in cotton and soybean fields in 2019. This is nearly 10 times the amount used in these cropping systems prior to 2017. The state of Tennessee accounts for approximately 5% of this dicamba use, despite planting fewer hectares in soybean and cotton compared with other states. Overreliance on a specific herbicide site of action can lead to increased selection for herbicide-resistant biotypes (Beckie and Rebound 2009; Powles et al. 1997).

In 2019, Tennessee growers began reporting to the Extension Service that dicamba was no longer controlling Palmer amaranth in their fields. A preliminary field screen for dicamba resistance was conducted at a grower's field in Lauderdale County in 2020 and 2021. Additionally, in 2020, a field screen was conducted in Gibson County. The herbicide had been applied when Palmer amaranth reached 10 cm in height. Treatments consisted of dicamba applied at 0.56 (1 $\times$ ) and 1.12 (2 $\times$ ) kg ae ha<sup>-1</sup>. Dicamba was applied using a CO<sub>2</sub>-pressurized backpack sprayer equipped with Turbo TeeJet Induction 11002 (TeeJet® Technologies, Glendale Heights, IL) nozzles calibrated to deliver 140 L ha<sup>-1</sup> at 4.8 kph using 220 kPa. In addition, a preliminary greenhouse screen of Palmer amaranth from Lauderdale County (two sites) and Gibson County, TN, was conducted in spring 2020. In that screen, dicamba was applied as described earlier but only at one rate (0.56 kg ha<sup>-1</sup>) to 10-cm-tall Palmer amaranth. In each of these

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**Table 1.** Palmer amaranth accessions screened for dicamba resistance.

Location	Coordinates	
	°N	°W
Gibson 1	35.7889	88.7967
Madison 1	35.7849	88.9171
Crockett 1	35.8262	89.0456
Carroll	35.9221	88.6462
Crockett 2	35.7816	89.1327
Madison 2	35.6321	88.8557
Lauderdale 1	35.7123	89.9175
Gibson 2	35.7815	88.8516
Lauderdale 2	35.7204	89.8771
Shelby	35.3421	89.8051
Dyer	36.0701	89.534
Tipton	35.6204	89.6151
Gibson 3	35.87	89.0458
Lauderdale 3	35.7183	89.8544
Lauderdale 4	35.7158	89.9187

screens, control of Palmer amaranth was  $\leq 50\%$  following timely applications of dicamba, prompting the larger survey and dose-response experiment.

A survey and seed collection of Palmer amaranth escapes in dicamba-resistant cotton and soybean fields in Tennessee was conducted in fall 2021 with the objective of determining whether poor control was due to an environmental phenomenon or to the development of dicamba-resistant Palmer amaranth in western Tennessee.

## Materials and Methods

A greenhouse dose-response experiment was conducted in 2021 and 2022 at the West Tennessee AgResearch and Education Center in Jackson, TN (35.632003°N, 88.855874°W). Palmer amaranth seeds from 15 locations in western Tennessee where dicamba failures were reported were collected in fall 2021 (Table 1). A specific field history for most individual commercial field locations was unknown; however, extensive use of dicamba for the past two decades in burndown due to widespread no-till practices across the state and more recently in-crop use in Xtend® crops suggests heavy dicamba use regardless of location (USDA-NASS 2018). The specific field history for the Lauderdale County 1 location was known and consisted of Xtend® cotton planted from 2016 to 2021. The Gibson County 1 and 2 sites were planted with Xtend® cotton from 2016 to 2020, Enlist® cotton in 2021, and Xtend® cotton again in 2022. In 2019, both growers noticed a small area of escaped Palmer amaranth after multiple applications of dicamba at 0.56 kg ae ha<sup>-1</sup>. Seeds were collected from these fields after being brought to the authors' attention by extension agricultural agents or crop consultants, and a preliminary greenhouse screen for dicamba resistance was conducted in 2020 prior to the survey at hand (results not shown).

Seeds from all 15 survey sites were processed and stored at 4 C for 4 wk before greenhouse trials were initiated. A known susceptible population of Palmer amaranth purchased from Azlin Seed Services (Leland, MS) was included for comparison. Palmer amaranth seeds were sprinkled on top of premoistened potting mix (Sta-Green Moisture Max Potting Mix) in 28 cm by 55 cm by 6 cm greenhouse trays (Greenhouse Megastore, Danville, IL).

Seeds were covered with 0.5 cm of potting mix and received overhead watering. Trays were kept moist throughout the experiment using subsurface irrigation, and supplemental lighting was used to ensure a 16-h photoperiod; daytime temperature was set to 33 C, and nighttime temperature was 26 C. Once plants emerged, Palmer amaranth plants were thinned to one plant per 30 cm<sup>2</sup>, or approximately 50 per tray. Trays were arranged in a randomized complete block design, and each tray was considered one plot, or experimental unit. The experiment was repeated two times with three replications, or trays, per population in each run.

Herbicide treatments were applied using a stationary greenhouse spray chamber (Devries Manufacturing, Hollandale, MN) calibrated to deliver 140 L ha<sup>-1</sup> at 4.8 kph using 200 kPa from a boom set up with two Turbo TeeJet Induction 11002 (TeeJet® Technologies) nozzles. The herbicide was applied when Palmer amaranth plants reached 10 cm in height. Treatments consisted of dicamba (Xtendimax® with VaporGrip® Technology; Bayer CropScience, St. Louis, MO) applied at 0.14 (0.25×), 0.28 (0.5×), 0.56 (1×), and 1.12 (2×) kg ae ha<sup>-1</sup>. The 1× rate was based on the XtendiMax label in which 0.56 kg ha<sup>-1</sup> (Anonymous 2022) is designated as the labeled over-the-top use rate for tolerant cotton and soybeans. Plants were placed in the greenhouse after application and grown for 21 d, after which the number of dead and live plants per flat were counted to calculate a percent mortality (control) and fresh weight of surviving plants was measured in grams.

Percent control and fresh weights were subjected to ANOVA using the GLIMMIX procedure in SAS software, version 9.4 (SAS Institute, Cary, NC) with Tukey's honestly significant difference at  $\alpha = 0.05$  for means separation. Location, herbicide rate, and location\*herbicide rate interactions were tested for significance. Single degree-of-freedom contrast statements were conducted to compare each suspected resistant accession to the susceptible check-by rate. Percent control was fit to a three-parameter sigmoidal curve using SigmaPlot 14.5 (Systat Software Inc, San Jose, CA) as suggested by Thornley and Johnson (1990), where Parameter a described the upper limit of control, Parameter b estimates the slope, and Parameter c represents the EC<sub>50</sub> rate (Equation 1). The EC<sub>50</sub> value was then subjected to ANOVA using the same methodology as the percent control and fresh weight values. Both replication and run were considered random effects in the model. Relative resistance factor was then calculated by dividing the EC<sub>50</sub> estimate for each population by the EC<sub>50</sub> estimate for the susceptible population.

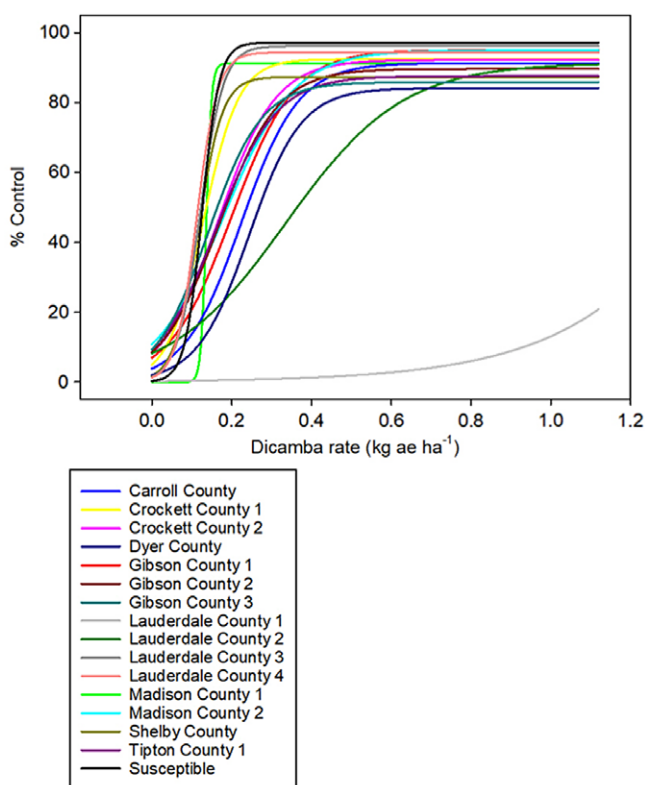
$$y = a / \{1 + \exp[-(\text{rate} - c)/b]\} \quad [1]$$

## Results and Discussion

Contrast statements used to compare the response of 15 Palmer amaranth accessions from Tennessee to a known susceptible check following increasing rates of dicamba showed a decrease in control at 0.14 kg ae ha<sup>-1</sup> for 10 of 15 accessions (Table 2). Four Tennessee accessions (Carroll, Lauderdale 1, Lauderdale 2, and Dyer counties) were not effectively controlled at the 0.28 kg ae ha<sup>-1</sup> rate. When using the 1× field rate (0.56 kg dicamba ha<sup>-1</sup>), Lauderdale 1 (1%), Lauderdale 2 (72%), Tipton (81%), and Gibson 3 (80%) County accessions exhibited less control than the susceptible check (95%). At 1.12 kg ae ha<sup>-1</sup>, dicamba controlled Palmer amaranth by 20%, 79%, and 82% at Lauderdale 1, Madison 1, and Dyer counties, respectively, while control for the susceptible check was 100%.

**Table 2.** Contrast statements comparing percent Palmer amaranth mortality of 15 accessions with a susceptible accession following increasing rates of dicamba.

Location	0.14 kg dicamba ha <sup>-1</sup>		0.28 kg dicamba ha <sup>-1</sup>		0.56 kg dicamba ha <sup>-1</sup>		1.12 kg dicamba ha <sup>-1</sup>	
	% Mortality	P-value	% Mortality	P-value	% Mortality	P-value	% Mortality	P-value
Gibson 1	35	0.0005	68	0.1295	91	0.3877	98	0.8453
Madison 1	60	0.3181	92	0.4823	100	0.8743	79	0.0372
Crockett 1	54	0.1077	82	0.8039	92	0.4577	99	0.9200
Carroll	25	<.0001	62	0.0432	89	0.2681	93	0.4015
Crockett 2	44	0.0091	72	0.2297	100	0.8743	88	0.1765
Madison 2	47	0.0186	66	0.0869	96	0.7704	95	0.5882
Lauderdale 1	5	<.0001	3	<.0001	1	<.0001	20	<.0001
Gibson 2	43	0.0075	69	0.1430	93	0.4847	89	0.2057
Lauderdale 2	21	<.0001	41	0.0002	72	0.0014	92	0.3702
Shelby	61	0.3331	77	0.5064	91	0.3779	100	0.9748
Dyer	15	<.0001	54	0.0052	85	0.1206	82	0.0444
Tipton	45	0.0128	68	0.1161	81	0.0310	97	0.7246
Gibson 3	50	0.0412	68	0.1258	80	0.0263	97	0.7218
Lauderdale 3	64	0.5381	91	0.5845	98	0.9953	100	0.9819
Lauderdale 4	70	0.5548	84	0.2781	99	0.6997	100	1.0000
Susceptible check	64		96		95		100	

**Figure 1.** Dicamba dose response of 15 Tennessee accessions. The responses of Palmer amaranth to increasing rates of dicamba as described by Equation 1:  $y = a/[1 + \exp\{-(-rate-c)/b\}]$  in which Parameter a described the upper limit of control, Parameter b estimates the slope, and Parameter c represents the EC<sub>50</sub> rate.

Dicamba dose-response curves suggest that Palmer amaranth populations in Tennessee are segregating based on their relative susceptibility to dicamba (Figure 1). Eight accessions responded with higher tolerance or resistance to dicamba compared with the known susceptible check. Of those eight accessions, three showed less control at rates two to four times above the 0.56 kg ha<sup>-1</sup> rate. The Lauderdale County 1 accession represented by the grey line showed an order of magnitude greater resistance to dicamba than all other accessions.

The EC<sub>50</sub> value for the susceptible check was 0.1262, indicating that this amount of dicamba per hectare would control 50% of the population (Table 3). Four Tennessee Palmer amaranth accessions had higher EC<sub>50</sub> values than the susceptible check: Carroll County (0.2338), Lauderdale County 1 (1.7978), Lauderdale County 2 (0.3140), and Dyer County (0.2398). The relative resistance factor for Carroll, Lauderdale 2, and Dyer counties was between 1.85 and 2.49, whereas the relative resistance factor for the Lauderdale County 1 accession was 14.25, indicating that this population has developed a high level of resistance and can no longer be effectively controlled using dicamba. These results are consistent with reports from the grower who manages this field. Lauderdale and Tipton counties in Tennessee have been the epicenter for Palmer amaranth resistance to herbicides in previous years and is where one of the first Palmer amaranth populations that have resistance to glyphosate and protoporphyrinogen oxidase were discovered in the state (Copeland et al. 2018; Steckel et al. 2008).

Fresh weight of surviving plants was measured 21 d after application. At less than 0.56 kg ae ha<sup>-1</sup> of dicamba, an increase in biomass was observed in some accessions compared with the nontreated control of those same accessions. Because the location\*rate interaction was not significant for fresh weights, but location was significant, fresh weight was averaged for each location and compared to the susceptible check (Table 4). Lauderdale County 1 (106%) and Carroll County (40%) were the only accessions to exhibit higher overall biomass as a percent of the nontreated control compared with the susceptible check (20%). These findings support the control results with the Lauderdale County 1 accession showing an actual biomass increase after a dicamba application compared to the same accession that was not treated.

These data document a segregating population of Palmer amaranth to dicamba in Tennessee. It ranges from 11 accessions with control similar to the susceptible check to three accessions (Carroll, Dyer, Lauderdale 2) showing resistance ratios of 1.85 to 2.49. The Lauderdale 1 accession is confirmed to be highly resistant with a resistant ratio of 14.25. Another step to confirm resistance is documenting heritability of the resistance between generations. The history of Palmer amaranth escaping dicamba in the Lauderdale 1 location from 2019 to 2021 in the grower's field, preliminary field research, and this greenhouse dose response would indicate that the dicamba resistance has passed between generations. This demonstrates the dicamba resistance allele or

**Table 3.** Response of Tennessee Palmer amaranth accessions to dicamba in 2022.

Location	EC <sub>50</sub> <sup>a,b</sup>	RRF
	kg ae ha <sup>-1</sup>	
Gibson 1	0.1945 cd	1.55
Madison 1	0.1301 d	1.03
Crockett 1	0.1312 d	1.04
Carroll	0.2338 bc	1.85
Crockett 2	0.1776 cd	1.41
Madison 2	0.1792 cd	1.42
Lauderdale 1	1.7978 a	14.25
Gibson 2	0.1638 cd	1.30
Lauderdale 2	0.3140 b	2.49
Shelby	0.1246 d	0.98
Dyer	0.2398 bc	1.90
Tipton	0.2063 cd	1.64
Gibson 3	0.1676 cd	1.33
Lauderdale 3	0.1210 d	0.96
Lauderdale 4	0.1133 d	0.90
Susceptible check	0.1262 d	1

<sup>a</sup>Abbreviations: EC<sub>50</sub>, half-maximal effective concentration; RRF, relative resistance factor.

<sup>b</sup>Means not followed by a common letter are significantly different ( $P < 0.05$ ).

**Table 4.** Palmer amaranth accession fresh weights 21 d following dicamba application.

Location	Fresh weight	
	Percent of nontreated <sup>a</sup>	
Gibson 1	23 c	
Madison 1	18 c	
Crockett 1	21 c	
Carroll	40 b	
Crockett 2	25 c	
Madison 2	24 c	
Lauderdale 1	106 a	
Gibson 2	23 c	
Lauderdale 2	30 bc	
Shelby	32 bc	
Dyer	26 c	
Tipton	23 c	
Gibson 3	31 bc	
Lauderdale 3	21 c	
Lauderdale 4	20 c	
Susceptible check	20 c	

<sup>a</sup>Means not followed by a common letter are significantly different ( $P < 0.05$ ).

alleles were passed from the 2019 Palmer amaranth generation to the 2020 and the 2021 generations. This research documents the first findings of Palmer amaranth control failures in cotton and soybean fields due to the evolution of dicamba resistance.

Dicamba resistance in Palmer amaranth greatly limits control options in cotton and soybean. Glyphosate-resistant Palmer amaranth was first documented in Tennessee in 2008 (Steckel et al. 2008). By 2013, the glyphosate-resistant biotype had become predominant in western Tennessee and was becoming established in central Tennessee (Steckel 2013). Recent documentation of glufosinate-resistant Palmer amaranth (Priess et al. 2022) in the Arkansas county adjacent to Lauderdale County, Tennessee, calls into question whether the XtendFlex trait (Bayer CropScience, St. Louis, MO) that provides cotton and soybean resistance to

dicamba, glyphosate, and glufosinate will be a viable weed management tool for this weed in future years.

Future research should be conducted to determine whether dicamba-resistant Palmer amaranth accessions are cross-resistant to 2,4-D. In addition, research designed to assess the mechanism or mechanisms of resistance with the Lauderdale County 1 accession will be conducted. Finally, weed management research needs to be conducted to determine how best to integrate herbicides and nonchemical tactics to better control Palmer amaranth.

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