Weed Technology

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Response of dicamba-resistant Palmer amaranth and cotton to malathion applied in conjunction with dicamba

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Research Article

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Abstract

Cotton and soybean growers were offered new technologies in 2016, expanding in-crop herbicide options to include dicamba or 2,4-D. Within 3 yr of commercialization, dicamba use in these crops increased 10-fold, and growers began to report Palmer amaranth escapes in dicamba-tolerant production systems in western Tennessee. In 2020, Palmer amaranth seed was collected from eight Tennessee locations where growers witnessed poor control following dicamba. Greenhouse experiments were conducted to evaluate the response of these Palmer amaranth populations to dicamba. In 2021, field experiments were conducted on two tentative dicamba-susceptible populations in Georgia, on three confirmed dicamba-resistant populations in Tennessee, and on a tentative dicamba-susceptible population in Texas to evaluate cotton response following dicamba and to examine if malathion insecticide (a cytochrome P450 inhibitor) would improve weed control and not reduce cotton yield when applied in conjunction with dicamba. Palmer amaranth populations collected in 2020 survived dicamba in the greenhouse at 1, 2, and 4 times the labeled rate. Five Palmer amaranth populations exhibited 15% to 26% survival to the labeled dicamba rate (560 g ha^{-1}) in the greenhouse. These findings were reinforced in the field when research on three of those populations in 2021 showed 55% control with the labeled dicamba rate and 69% control with 2 times the labeled rate. This demonstrates that the dicamba resistance allele or alleles were passed between generations. This result was not consistent in the Macon County, GA, or Worth County, GA, locations, where malathion improved dicamba control of 15- to 38-cm-tall Palmer amaranth. Cotton injury was observed when malathion was applied in combination with dicamba. These results further document the evolution of dicamba-resistant Palmer amaranth in Tennessee. Moreover, the nonreversal of resistance phenotype by malathion may suggest that the resistance mechanism is something other than metabolism.

Introduction

Palmer amaranth originated in the dry southwestern United States and Mexico but is now present across the entire southern United States and in some northern states, such as Illinois and Minnesota (Sauer 1950; Steckel 2007). In a 2019 survey by the Weed Science Society of America (WSSA), Palmer amaranth ranked as the most common and most troublesome weed species among all broadleaf crops, fruits, and vegetables (Van Wychen 2019). Since its first known case of herbicide resistance in 1989, Palmer amaranth has evolved resistance to herbicides from eight modes of action, including acetolactate synthase (ALS) inhibitors (WSSA Group 2), auxin mimics (WSSA Group 4), 5-enolpyruvylshikimate-3-phosphate synthase inhibitors (WSSA Group 9), 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors (WSSA Group 27), microtubule assembly inhibitors (WSSA Group 3), photosystem II binders (WSSA Group 5), protoporphyrinogen oxidase (PPO) inhibitors (WSSA Group 14), and long-chain fatty acid inhibitors (WSSA Group 15) (Heap 2022). Most recently, Foster and Steckel (2022) confirmed dicamba-resistant Palmer amaranth in Tennessee.

Herbicides are the most effective and economical approach to control weeds in cotton and soybean, but overreliance on limited modes of action has resulted in the rapid evolution of herbicide-resistant weeds (Young 2006). Cotton and soybean growers were offered new technologies in 2016, expanding in-crop herbicide options, including dicamba and 2,4-D, but these technologies are not without their own resistance-development challenges. The first documented case of dicamba resistance in the United States was in kochia [*Kochia scoparia* (L.) Schrad.] in 1994 (Heap 2022). To date, kochia, prickly lettuce (*Lactuca serriola* L.), and Palmer amaranth have been confirmed with dicamba resistance in the United States (Heap 2022). With a longer history of use, several weed species have been confirmed resistant to 2,4-D. In 1957, spreading dayflower (*Commelina diffusa* Burm. f.) became the first herbicide-resistant weed in the United States, conferring resistance to 2,4-D (Heap 2022). Today, five other weed species in the United States have evolved resistance to 2,4-D, including Palmer amaranth, tall waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer], buckhorn plantain (*Plantago lanceolata* L.), wild carrot (*Daucus carota* L.), and prickly lettuce (Heap 2022).

In western Tennessee, where row crop production averages 1.3 million ha each year, more than 94,000 kg of dicamba have been applied annually from 1992 to 2019 (USGS 2021). This is mainly because 85% of Tennessee cotton, corn (*Zea mays* L.), and soybean hectares are being farmed using no-till production, relying on dicamba as part of the preplant burndown program for decades (USDA-NASS 2018). Much of central Tennessee is encompassed by pastures, where 2,4-D has been used extensively year after year at a rate of >49 g ha^{-1} annually for the past two decades (USGS 2021). While historical use of dicamba and 2,4-D likely contributes to resistance issues, the use of these herbicides has increased 10-fold in cotton and soybean production since the commercialization of tolerant varieties in 2016. Tillage is much more extensively used in row crop production in Georgia and Texas than it is in Tennessee, which has led to less reliance on auxin herbicides prior to planting (USDA-NASS 2018; USGS 2021).

Cytochrome P450s are a class of enzymes belonging to the largest family involved in oxygen-dependent hydroxylation reactions (Pandian et al. 2020). Cytochrome P450s are present in most life processes and are one of the main contributors to oxidation-based metabolism in plants (Mizutani and Sato 2011). These enzymes, specifically cytochrome P450 monooxygenase, contribute to herbicide detoxification by adding an oxygen atom onto herbicide structures to make them more hydrophilic, thereby facilitating easier degradation in subsequent metabolic reactions; overexpression of just one of the many cytochrome P450 genes can confer herbicide resistance (Hirose et al. 2005; Hu et al. 2009). Indeed, the dicamba resistance in Xtend® (Bayer Crop Science, St. Louis, MO, USA) cotton is garnered from dicamba monooxygenase, a cytochrome P450 (Behrens et al. 2007).

Interactions between certain cytochrome P450–inhibiting organophosphate insecticides with herbicides have been shown to increase crop injury in cases in which crop tolerance was mediated by metabolism of the herbicide. In corn, many herbicide labels restrict the use of these insecticides to be used in conjunction with one another; two examples are Accent® Q (Corteva Agriscience, Indianapolis, IN, USA) and HALEX® GT (Syngenta Crop Protection, Greensboro, NC, USA) (Anonymous 2022a, 2022b). This was documented initially by Kapusta and Krausz (1992), who determined that the insecticide terbufos increased corn yield but had an adverse effect on yield when applied in close timing with nicosulfuron. More recent research has shown that foliar or in-furrow applications of the organophosphate insecticide chlorpyrifos increased injury and decreased grain yield when used in conjunction with HPPD inhibitor–based premixed herbicides in corn (Steckel et al. 2015).

Previous research has suggested that organophosphate insecticides (such as malathion) are good candidates to test possible nontarget-site metabolic-resistance mechanisms in weeds (Kumar et al. 2020; Varanasi et al. 2019). Cytochrome P450 inhibitors prevent the hydroxylation of herbicides by P450 enzymes, thereby slowing degradation and increasing the half-life of the herbicide within plants (Kreuz and Fonne-Pfister 1992). Shyam et al. (2020) reported that malathion applications 30 min prior to 2,4-D applications reversed 2,4-D resistance in Palmer amaranth. In recent years, several news articles on the use of organophosphate insecticides for mitigating metabolic-driven (cytochrome P450 based) herbicide resistance have been published (Benjamin 2017).

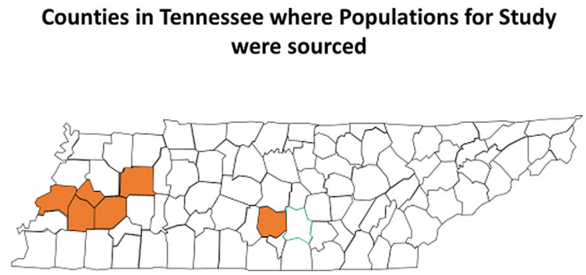

Figure 1. Tennessee counties, in orange, where Palmer amaranth populations were collected in fall 2020 to determine potential resistance to dicamba in the greenhouse.

The objectives of this research were to (1) examine if eight Tennessee, two Texas, and two Georgia Palmer amaranth populations have evolved dicamba resistance; (2) explore the role of malathion (cytochrome P450 inhibitor) in conjunction with dicamba applications for managing dicamba-resistant Palmer amaranth; and (3) evaluate cotton response to malathion applied in conjunction with dicamba.

Materials and Methods

Greenhouse Experiments

Seed Source

Palmer amaranth seed was collected in fall 2020 from eight Tennessee locations in six counties (Figure 1) and from two Texas locations in one county (Figure 2), as noted in Table 1. The background of these Tennessee fields where the seed was collected consisted of extensive use of dicamba for the previous 2 decades in burndown before planting and more recently, when dicamba was used in Xtend® crops (USDA-NASS 2018). All Palmer amaranth populations were sourced from a 65-km radius of Jackson, TN, except for the Bedford County, TN, population, which was 240 km from Jackson, TN. The Bedford County field was planted to Xtend® soybean from 2017 to 2019, when the growers observed very poor control of 8- to 14-cm Palmer amaranth with 0.56 kg ha^{-1} of dicamba. The field site in Madison County, TN, was planted to dicamba-resistant soybean from 2017 through 2020. In 2020, very poor control (<60%) occurred with two applications of dicamba applied at a rate of 0.56 kg ha^{-1} (data not shown). The other six sites were all planted to Xtend® cotton from 2017 to 2020. Growers managing these sites began experiencing poor Palmer amaranth control with dicamba in either 2019 or 2020. Specifically, seed was collected from Palmer amaranth escapes in 2019 at the Bedford County, TN; Gibson County, TN 2; and Crockett County, TN 1 and TN 2, locations in fall 2019 and underwent a preliminary greenhouse screen for resistance to dicamba. Greenhouse screening results showed more than 10% survivors from these locations following dicamba at the 0.56 kg ha^{-1} rate. In 2020, research was conducted in the field using 0.56 kg ha^{-1} and 1.12 kg ha^{-1} dicamba rates on 5- to 10-cm-tall Palmer amaranth at the Gibson County, TN 2, and Crockett County, TN 1, locations. In those studies, only 50% and 60% Palmer amaranth control was acquired with those two respective rates. Subsequent greenhouse and field research at

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Figure 2. Lubbock County, Texas, in orange, where Palmer amaranth populations were collected in fall 2020 to determine potential resistance to dicamba in the greenhouse.

the Gibson County, TN 2, location showed that the population has a relative resistance factor of 1.55 (Foster and Steckel 2022). The Texas populations are still effectively controlled by dicamba and have not endured as much selection pressure for dicamba resistance to develop as the Tennessee populations have (USGS 2021).

Greenhouse Study

In 2021, a greenhouse experiment was conducted four times at the Texas A&M AgriLife Research Center in Lubbock, TX (33.692°N, 101.823°W). Seeds were sprinkled on top of moist potting mix (BM6; Berger, Edmond, OK, USA) in pots with 11.4 × 11.4 × 9.5 cm dimensions; seeds were lightly covered with potting mix and watered. Pots were placed on benches with supplemental lighting to ensure a daily photoperiod of 14 h. Daytime temperature setpoint was 32 C, and nighttime temperature was set to 27 C. Soil was kept moist with overhead irrigation until emergence, and seedlings were thinned to four to five plants per pot. Herbicide treatments were applied in a stationary greenhouse spray chamber equipped with Turbo TeeJet® induction nozzles (TeeJet® Technologies, Glendale Heights, IL, USA) calibrated to deliver 140 L ha^{-1} at 4.8 km h^{-1} using 200 kPa when plants reached a height of 8 to 10 cm. The experiment was replicated four times. Experimental design was a complete factorial (Malathion Timing × Dicamba Rate) within a randomized complete block. The first factor consisted of malathion at 2 kg ai ha^{-1} applied 24 h or 1 h before dicamba application, malathion mixed with dicamba, or a dicamba-alone control. The second factor was 0.56 (1X), 1.12 (2X), and 2.24 (4X) kg ae ha^{-1} rates of dicamba along with a nontreated.

The survival rate of Palmer amaranth was calculated by counting the number of dead and alive plants in each pot, and the fresh weight of live plants was measured 21 d after application. Data were analyzed using the GLIMMIX procedure in SAS (version 9.4; SAS Institute Inc., Cary, NC, USA) for analysis of variance (ANOVA) and Fisher's least significant difference (LSD) at $\alpha = 0.05$. Single degree of freedom contrast statements were conducted to compare each location to the known susceptible check that was sourced from Lubbock, TX. Finally, previous research suggested that Palmer amaranth populations from some of the Tennessee populations were 2X to 3X resistant dicamba (Foster and Steckel 2022). Overall, control of Palmer amaranth was better in the greenhouse than in the field. This is consistent with the findings of previous researchers who reported improved control with herbicides in the greenhouse compared with the field (Combellack 1982; Perkins et al. 2020). Because control in the greenhouse was better than that observed in the field, an alpha value of 0.1 was used for separation.

Field Experiments

Field experiments were conducted at three locations in Tennessee, two locations in Georgia, and one location in Texas. At all locations, a complete factorial (Malathion Timing × Dicamba Rate) within a

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Table 1. Location coordinates for Palmer amaranth populations collected in fall 2020 to determine potential resistance to dicamba in the greenhouse.

Location[a]	Latitude	Longitude
	°N	°W
Bedford County, TN	35.4415	86.6373
Carroll County, TN	36.0790	88.6824
Crockett County, TN 1	35.7814	89.1329
Crockett County, TN 2	35.7854	89.1567
Gibson County, TN 1	35.8702	89.0480
Gibson County, TN 2	35.7889	88.7964
Gibson County, TN 3	35.9668	89.0044
Lubbock County, TX	33.6896	101.8201
Lubbock County, TX 2	33.7311	101.7337
Madison County, TN	35.6310	88.8575

[a]All Palmer amaranth populations were sourced from a 65-km radius of Jackson, TN, except for the Bedford County population, which was 240 km from Jackson, TN.

randomized complete block study design was implemented. In Georgia and Tennessee, the first factor consisted of malathion at 2 kg ai ha^{-1} applied 24 h or 1 h before dicamba application, malathion mixed with dicamba, or a dicamba-alone control. The malathion rate was based on Shyam et al. (2020), who determined 2-kg ha^{-1} reversed auxin herbicide resistance in a Kansas population of Palmer amaranth. However, this rate is higher than what is recommended for row crops (Stewart et al. 2022). In Texas, treatment structure was identical, except there was no application 1 h before dicamba. The second factor at all locations was dicamba applied at 0.56 (1X), 1.12 (2X), and 2.24 (4X) kg ae ha^{-1} field rates and a nontreated control. All herbicides were applied using a CO_2-pressurized backpack sprayer equipped with TTI 11002 nozzles (TeeJet® Technologies) calibrated to deliver 140 L ha^{-1} at 4.8 kph using 220 kPa.

Tennessee field experiments were conducted in 2021 at three locations. The first location was the West Tennessee Agricultural Research and Education Center in Madison County (WTREC) (35.632°N, 88.856°W). The field history at this site is described previously. The second Tennessee location was a grower's field site in Madison County (35.782°N, 88.852°W). The field history consisted of Xtend® cotton planted from 2016 to 2019, followed by Enlist® cotton (Corteva Agriscience, Indianapolis, IN, USA) in 2020. The third location was a grower's field in Lauderdale County (35.715°N, 89.918°W). The field history was similar to that of the Madison County site, where Xtend® cotton was planted from 2016 through 2020. Greenhouse and field research confirmed dicamba resistance in these populations ranging from 1.85 to 14.25 relative resistance factor (Foster and Steckel 2022).

The WTREC site was equipped with lateral overhead irrigation, whereas the Madison County and Lauderdale County sites were rainfed (Table 2). Each location consisted of a native Palmer amaranth population with no crop present. Once the initial flush of Palmer amaranth emerged, pyroxasulfone at 0.12 kg ai ha^{-1} was applied over the study area to suppress new flushes of weeds. In addition, clethodim at 0.28 kg ai ha^{-1} was applied to control native junglerice [*Echinochloa colona* (L.) Link]. Plot size was 3 × 9 m. At the time of treatment application, Palmer amaranth was 10 cm in height.

Georgia field experiments were conducted in 2021 at a grower's field site in Macon County (32.423°N, 84.129°W), and at the University of Georgia's Coastal Plains Experiment Station Ponder Farm in Worth County (31.505°N, 83.651°W). The field history at both these sites was cotton weed control research, where dicamba had provided >80% Palmer amaranth control. Both sites were equipped with overhead irrigation. Deltapine® 1646B2XF cotton (Bayer Crop Science) was planted on April 27 at Macon County and on May 17 at Worth County with native Palmer amaranth populations present. Plot size was 2 × 8 m. Dicamba application was targeted for a crop stage of 3 to 5 leaves; therefore the height of Palmer amaranth at the time of dicamba application was 15 cm at Macon County and up to 38 cm at Worth County (Table 2). Palmer amaranth was much larger at both Georgia locations compared with Tennessee or Texas because of logistics and weather delay. At 7 d and 18 d after dicamba applications, *S*-metolachlor (1.0 kg ai ha^{-1}) was applied over the entire study area to prevent additional Palmer amaranth emergence. In addition, clethodim at 0.28 kg ha^{-1} was applied to control native annual grasses. Cotton production practices followed those for Georgia (Hand et al. 2022).

The field experiment in Texas was conducted at the Texas Tech University New Deal Research Farm in Lubbock County (33.731°N, 101.735°W). The field history at this site was cotton weed control research, where dicamba had provided >90% Palmer amaranth control. This site was equipped with subsurface drip irrigation. Deltapine® 1822XF was planted on June 5, 2021, and plots were maintained weed-free throughout the season using trifluralin at 1.12 kg ai ha^{-1} preplant incorporated, prometryn at 1.12 kg ai ha^{-1} preemergence, glyphosate at 1.4 kg ae ha^{-1} + glufosinate at 0.88 kg ai ha^{-1} + ammonium sulfate at 3.4 kg ha^{-1} postemergence, cultivation, and hand-weeding (Table 2). Treated plot size was 2 × 9 m. Cotton was 8-leaf at the time of treatment applications.

Palmer amaranth control at Tennessee and Georgia locations was evaluated visually using a 0% to 100% scale (Frans et al. 1986) and by counting the number of surviving plants per square meter at 14 to 28 d after treatment (DAT) with dicamba. Cotton injury was rated on a 0% to 100% scale, with 0% indicating no injury and 100% indicating complete crop death at 5, 9, and 14 DAT at Texas and Georgia locations. Cotton lint yield was collected per plot using a spindle picker at the Georgia locations and a cotton stripper at New Deal. Data were analyzed using the GLIMMIX procedure in SAS for ANOVA and Fisher's LSD at $\alpha = 0.05$.

Results and Discussion

Greenhouse Experiment

In the greenhouse experiment, malathion did not influence Palmer amaranth control regardless of dicamba rate. Thus dicamba rate by population was the only factor evaluated when determining Palmer amaranth response. When dicamba was applied at 0.56 kg ha^{-1}, five Tennessee Palmer amaranth populations had a greater survival percentage when compared with the known susceptible Lubbock County, TX 1, population (Table 3). These Tennessee populations with greater survival percentages were Crockett County, TN 1 (15%) ($P = 0.0997$) and 2 (18%) ($P = 0.0324$); Bedford County, TN (26%) ($P = 0.0021$); Gibson County, TN 2 (18%) ($P = 0.0371$); and Carroll County, TN (16%) ($P = 0.0730$). At 1.12 kg ha^{-1} of dicamba, the Crockett County, TN 1 (14%) ($P = 0.0461$), and Madison County, TN (13%) ($P = 0.0571$), populations had a greater percentage of survivors when compared with Lubbock County, TX 1 (2%).

Palmer amaranth fresh weight as a percentage of the nontreated control showed similar results, where greater weights were recorded in the Bedford County, TN (16%) ($P = 0.0765$), and Carroll County, TN (18%) ($P = 0.0215$), populations when compared with Lubbock County, TX 1 (8%), following dicamba at 0.56 kg ae ha^{-1} (Table 4).

Table 2. Application details for field experiments in Georgia, Tennessee, and Texas.

Location	County	Crop	Crop stage at application	Palmer amaranth size	Irrigation or rainfed
				cm	
WTREC	Madison County, TN	Noncrop	N/A	10	Lateral overhead irrigation
Madison	Madison County, TN	Noncrop	N/A	10	Rainfed
Lauderdale	Lauderdale County, TN	Noncrop	N/A	10	Rainfed
Macon County	Macon County, GA	Cotton	3- to 5-leaf	15	Pivot overhead irrigation
Ponder Farm	Worth County, GA	Cotton	3- to 5-leaf	38	Lateral overhead irrigation
New Deal	Lubbock County, TX	Cotton	8-leaf	Weed-free	Subsurface drip irrigation

Table 3. Contrast statements comparing survival rate of Palmer amaranth populations versus a known susceptible population (Lubbock County, TX 1) following increasing rates of dicamba.

	0.56 kg ha^{-1}		1.12 kg ha^{-1}		2.24 kg ha^{-1}	
Location	Survival rate	P-value	Survival rate	P-value	Survival rate	P-value
	%		%		%	
Lubbock County, TX 1	3		2		5	
Bedford County, TN	26	0.0021	8	0.3398	4	0.8090
Carroll County, TN	16	0.0730	9	0.2073	4	0.8123
Crockett County, TN 1	15	0.0997	14	0.0461	1	0.1902
Crockett County, TN 2	18	0.0324	5	0.5990	3	0.5926
Gibson County, TN 1	12	0.3039	8	0.3217	7	0.5772
Gibson County, TN 2	18	0.0371	7	0.4001	0	0.1281
Gibson County, TN 3	9	0.4327	6	0.4654	1	0.1918
Lubbock County, TX 2	10	0.3481	7	0.4174	3	0.4576
Madison County, TN	7	0.5934	13	0.0571	1	0.1783

Table 4. Contrast statements comparing fresh weight of Palmer amaranth populations versus a known susceptible population (Lubbock County, TX 1).

	0.56 kg ha^{-1}		1.12 kg ha^{-1}		2.24 kg ha^{-1}	
Location	Fresh weight[a]	P-value	Fresh weight	P-value	Fresh weight	P-value
	%		%		%	
Lubbock County, TX 1	8		8		9	
Bedford County, TN	16	0.0765	4	0.0725	5	0.0051
Carroll County, TN	18	0.0215	10	0.1305	8	0.3147
Crockett County, TN 1	8	0.9329	7	0.6502	3	<0.0001
Crockett County, TN 2	8	0.9606	5	0.1113	5	0.0063
Gibson County, TN 1	12	0.4792	6	0.5564	6	0.0263
Gibson County, TN 2	15	0.1274	6	0.2636	5	0.0009
Gibson County, TN 3	12	0.3345	8	0.5726	7	0.1696
Lubbock County, TX 2	15	0.1239	10	0.1377	7	0.1700
Madison County, TN	8	0.8845	10	0.2104	6	0.0437

[a]Expressed as percentage of the nontreated control.

Field Experiments

Weed Control Studies

Weed control studies were separated by location in Georgia, with differing results likely influenced by Palmer amaranth size at the time of application. No differences among locations were noted in Tennessee, and data were combined across three locations.

Palmer amaranth was controlled 73%, 92%, and 97% with the 1X, 2X, and 4X rate of dicamba, respectively, at Macon County, GA, at 28 DAT (Table 5). The addition of malathion increased Palmer amaranth control by 10% to 16% when dicamba was applied at the 0.56 kg ae ha^{-1} rate. Higher use rates of dicamba provided at least 92% control; thus the benefit from malathion likely would not be detectable. Palmer amaranth density 14 DAT was almost 74,000 plants ha^{-1} in the nontreated control. Dicamba alone at the 1X rate decreased density by 82%, and the addition of malathion 24 h or 1 h before dicamba further decreased Palmer amaranth density by 89% to 90% of the nontreated control. Malathion timing did not further decrease density when dicamba was applied at 1.12 or 2.24 kg ha^{-1}.

At Worth County, GA, Palmer amaranth control increased by 15% to 25% when malathion was applied 1 h before or in mixture with the 1X rate of dicamba (Table 5). At the 1.12 kg ha^{-1} dicamba rate (2X), malathion applied 24 h or 1 h before the herbicide increased Palmer amaranth control 9% to 12% compared with dicamba alone. All dicamba–malathion combinations at the 2.24 kg ae ha^{-1} dicamba rate were similar to the herbicide applied alone. Palmer amaranth density at Worth County was 17,346 plants ha^{-1} in the nontreated control 28 DAT. Only the addition of malathion applied 1 h before dicamba at 0.56 kg ha^{-1} decreased Palmer amaranth density compared with the same rate of the herbicide applied alone.

In Tennessee, Palmer amaranth control was not affected by malathion timing but was influenced by dicamba rate. Palmer

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Table 5. Palmer amaranth control 28 d following dicamba applications with and without malathion.[a,b]

Dicamba rate	Malathion timing	Macon County, GA		Worth County, GA, 28 DAT		Tennessee[c]	
		28 DAT	14 DAT			28 DAT	21 DAT
kg ae ha^{-1}		%	plants ha^{-1}	%	plants ha^{-1}	%	plants ha^{-1}
Nontreated control		N/A	73,900 a	N/A	17,300 a	N/A	166,700 a
0.56	No malathion	73 f	13,300 b	56 f	4,100 b	55 c	156,100 ab
	24 h	86 de	8,200 c	76 c–e	3,600 bc	57	85,600 cd
	1 h	89 cd	7,500 c	81 b–d	2,300 ef	56	110,300 a–c
	With dicamba	83 e	14,300 b	71 e	4,200 b	55	99,700 b–d
1.12	No malathion	92 bc	3,800 cd	75 de	2,800 c–e	69 b	73,200 cd
	24 h	95 ab	3,400 cd	84 bc	2,700 c–e	70	106,700 a–d
	1 h	94 a–c	2,367 d	87 ab	1,900 ef	70	90,900 cd
	With dicamba	93 a–c	5,300 cd	81 b–d	3,600 b–d	69	82,100 cd
2.24	No malathion	97 ab	900 d	88 ab	2,600 d–f	85 a	60,900 cd
	24 h	99 a	800 d	93 a	2,000 ef	83	45,000 d
	1 h	99 a	800 d	93 a	1,700 f	87	45,000 d
	With dicamba	98 a	1,563 d	89 ab	2,504 ef	84	46,794 d
P-values							
Rate		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Malathion timing		0.0021	0.0144	<0.0001	0.0056	0.5492	0.1166
Rate × Malathion Timing		0.0052	<0.0001	0.0587	<0.0001	0.9705	0.0009

[a]Percentages followed by the same letter are not different according to Tukey's honestly significant difference ($\alpha = 0.05$).
[b]Abbreviations: DAT, days after treatment; N/A, not applicable.
[c]Tennessee data are combined across three locations.

amaranth was controlled 55%, 69%, and 85% with the 1X, 2X, and 4X rate of dicamba, respectively (Table 5). Palmer amaranth density in the nontreated control was >166,000 plants ha^{-1} 21 DAT. The labeled rate of dicamba (0.56 kg ha^{-1}) did not reduce Palmer amaranth populations compared with the nontreated control. Malathion applied 24 h prior to dicamba at the labeled rate was the only insecticide timing to decrease Palmer amaranth density (86,000 plants ha^{-1}). This result was similar to response shown by the Macon County, GA, Palmer amaranth population. The 1.12 kg ha^{-1} rate of dicamba reduced the Palmer amaranth population 53% compared with the 0.56 kg ha^{-1} rate. The 2.24 kg ha^{-1} rate did not reduce Palmer amaranth populations compared with the 1.12 kg ha^{-1} dicamba rate.

These results were consistent with reports of Palmer amaranth control failures by the Tennessee farmers who managed those fields. The Texas Palmer amaranth populations were susceptible to dicamba. This was consistent with extensive experience with the Texas population that showed good control with dicamba. As noted earlier, Palmer amaranth at application for the Georgia populations were quite large (15 to 38 cm); therefore weed size rather than dicamba resistance evolution may be the reason for poor control. Additional research on the Palmer amaranth at these sites would be warranted, as malathion did improve control.

Previous research by Shyam et al. (2020) indicated that 2,4-D resistance in Palmer amaranth was mitigated when malathion was mixed with 2,4-D and applied to the resistant biotype. In PPO-resistant Palmer amaranth populations, malathion followed by (fb) fomesafen or saflufenacil partially reversed resistance; however, malathion fb flumioxazin or acifluorfen had no effect on Palmer amaranth compared with those herbicides applied alone (Varanasi et al. 2018, 2019). Similarly, in ALS-resistant Palmer amaranth, 2,000 g ha^{-1} malathion applied 1 h before chlorsulfuron reduced plant biomass by 50% when compared with the herbicide applied alone (Nakka et al. 2017). Similar results have been reported in other weed species; for example, when malathion was mixed with imazamox and quinclorac and applied to resistant junglerice populations, resistance was mitigated (Wright et al. 2018).

Cotton-Response Field Experiments

Cotton-response field experiments were separated by location due to location interactions ($P < 0.0001$). In Macon County, GA, dicamba at the 1X, 2X, and 4X rate injured cotton 16%, 23%, and 41% at 5 DAT, respectively (Table 6). Applying malathion 24 h before dicamba did not influence injury compared with dicamba alone, regardless of rate. Malathion applied 1 h before dicamba applications increased crop injury when applied at the 2X and 4X rates, while the mixture showed 9% to 15% more injury at the 1X and 2X dicamba rate, respectively. Injury was generally similar at 9 DAT, but by 14 DAT, differences in injury were observed only when comparing dicamba rates with no effect from malathion. Cotton visual injury was not detectable by 21 DAT (data not shown). Plant height was measured, and malathion treatment had no influence on cotton height; however, there was a trend for shorter cotton when the 4X rate of dicamba was applied (data not shown). Cotton lint yield was not influenced by crop response or malathion but rather by Palmer amaranth control obtained with dicamba. Both higher use rates of dicamba noted higher yields when compared with the 1X dicamba rate; no yield was obtained from the nontreated control.

In Worth County, GA, malathion had less influence on cotton response from dicamba (Table 7). At 5 DAT, injury differences were observed only with increasing rates of dicamba ranging from 17% to 28%. By 9 DAT, injury response was similar to that observed at 5 DAT, except the mix of 1.12 kg ha^{-1} of dicamba with malathion was 9% more injurious than the respective rate of dicamba alone. By 14 DAT, visual injury was no longer detectable (data not shown). Plant height was measured, and herbicide treatment had no influence on cotton height; however, competition from Palmer amaranth decreased cotton height in the nontreated control late in the season (data not shown). Cotton lint yield was not influenced by malathion, dicamba rate, Palmer amaranth control among herbicide treatments, or crop response. Yield from the nontreated control was 0 kg ha^{-1}, and yields were improved by all herbicide treatments (721 to 1,115 kg ha^{-1}).

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Table 6. Cotton response to dicamba as influenced by malathion at Macon County, GA.[a,b]

Dicamba rate	Malathion timing	Cotton injury 5 DAT	Cotton injury 9 DAT	Cotton injury 14 DAT	Lint yield
kg ae ha^{-1}		%	%	%	kg ha^{-1}
0.56	No malathion	16 e	18 f	18 b	360 b
	24 h	19 de	19 ef	15	370
	1 h	24 de	26 cd	23	350
	With dicamba	25 d	21 d–f	16	370
1.12	No malathion	23 de	25 de	20 ab	520 a
	24 h	25 d	24 d–f	19	530
	1 h	40 bc	33 bc	21	500
	With dicamba	38 c	36 ab	21	560
2.24	No malathion	41 bc	35 ab	24 a	570 a
	24 h	46 ab	40 a	21	570
	1 h	50 a	40 a	28	500
	With dicamba	41 bc	35 ab	23	590
P-values					
Rate		<0.0001	<0.0001	0.0386	<0.0001
Malathion timing		<0.0001	0.0043	0.1887	0.3972
Rate × Malathion Timing		0.0264	0.0348	0.9500	0.7192

[a]Percentages followed by the same letter are not different according to Tukey's honestly significant difference ($\alpha = 0.05$).
[b]Abbreviation: DAT, days after treatment.

Table 7. Cotton response to dicamba as influenced by malathion at Worth County, GA.[a,b]

Dicamba rate	Malathion timing	Cotton injury 5 DAT	Cotton injury 9 DAT	Lint yield
kg ae ha^{-1}		%	%	kg ha^{-1}
0.56	No malathion	17 c	12 c	720
	24 h	16	15 bc	780
	1 h	16	16 bc	960
	With dicamba	18	14 c	930
1.12	No malathion	21 b	13 c	870
	24 h	23	13 c	990
	1 h	21	16 bc	1,120
	With dicamba	21	22 a	960
2.24	No malathion	28 a	24 a	930
	24 h	28	23 a	930
	1 h	28	23 a	1,040
	With dicamba	30	20 ab	990
P-values				
Rate		<0.0001	<0.0001	0.0681
Malathion timing		0.2413	0.298	0.0618
Rate × Malathion Timing		0.7813	0.023	0.9213

[a]Percentages followed by the same letter are not different according to Tukey's honestly significant difference ($\alpha = 0.05$).
[b]Abbreviation: DAT, days after treatment.

In Texas, cotton injury was greatest 5 DAT (Table 8). Dicamba at the 1X, 2X, and 4X rate injured cotton 10%, 18%, and 30%, respectively. Applying malathion 24 h before dicamba increased injury 8% to 12% with the 1X and 2X rates of dicamba. Malathion mixed with dicamba at the 1X, 2X, and 4X rate increased injury 16%, 9%, and 5%, respectively, compared with herbicide alone 5 DAT. Injury observations at 9 DAT were generally similar to those at 5 DAT. By 14 DAT, differences in injury were observed only when comparing dicamba rates. Injury was no longer visually detectable by 21 DAT (data not shown). Differences in cotton yield were not observed in this weed-free experiment.

Similar studies have shown that malathion applied in combination with other herbicides, such as pyrithiobac, increased cotton injury with no adverse effects on yield (Allen and Snipes 1995; Snipes and Seifert 2003; Minton et al. 2005). Postemergence applications of malathion mixed with trifloxysulfuron applied to 4- to 5-leaf cotton increased phytotoxicity 10% at 4 DAT compared with trifloxysulfuron alone (Minton et al. 2008). Insecticides in the same organophosphate class as malathion, such as dimethoate, produced similar results when applied in conjunction with pyrithiobac and glufosinate, where visual injury increased with the addition of the insecticide but cotton yield was not adversely affected (Costello et al. 2005; Steckel et al. 2012). Another possible explanation for the cotton injury is that dicamba resistance in cotton is derived with a P450 inhibitor, dicamba monooxygenase (Behrens et al. 2007). As such, the cotton injury could possibly be from the high rate of malathion deactivating dicamba monoxygenase, allowing dicamba to injure the cotton. However, the injury observed was a leaf burn, not the typical epinasty associated with dicamba. We suggest that the high rate of malathion acted like a surfactant, which caused necrosis of the leaf.

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Table 8. Cotton response to dicamba as influenced by malathion at New Deal, TX.[a,b]

		Cotton injury			
Dicamba rate	Malathion timing	5 DAT	9 DAT	14 DAT	Lint yield
kg ae ha^{-1}			%		kg ha^{-1}
Nontreated		N/A	N/A	N/A	1,410
0.56	No malathion	10 g	5 d	3 c	1,570
	24 h	22 e	19 c	6	1,400
	With dicamba	26 d	18 c	3	1,440
1.12	No malathion	18 f	16 c	10 b	1,380
	24 h	26 d	24 b	11	1,330
	With dicamba	27 cd	23 b	13	1,450
2.24	No malathion	30 bc	23 b	17 a	1,290
	24 h	32 ab	31 a	18	1,370
	With dicamba	35 a	34 a	15	1,320
P-values					
Rate		<0.0001	<0.0001	<0.0001	0.1796
Malathion timing		<0.0001	<0.0001	0.1991	0.7584
Rate × Malathion Timing		0.0007	0.0265	0.3424	0.4457

[a]Percentages followed by the same letter are not different according to Tukey's honestly significant difference (α = 0.05).
[b]Abbreviations: DAT, days after treatment; N/A, not applicable.

The recommended rate of malathion insecticide applied postemergence in cotton is 1.4 kg ai ha^{-1}, a lower rate than used in applications in these studies (Stewart et al. 2022). Although visual injury was observed, cotton lint yield was not reduced by the addition of malathion. In dicamba-susceptible Palmer amaranth populations, the addition of malathion to the labeled rate of dicamba improved control, but as herbicide rate increased, the benefit of adding malathion diminished. In dicamba-resistant Palmer amaranth populations, malathion did not improve control. Therefore this research suggests that adding malathion does not reverse dicamba resistance in Palmer amaranth and that the insecticide increased crop injury.

These results document dicamba failing to control Palmer amaranth sourced in 2020 in the greenhouse at 1, 2, and 4 times the labeled rate. Moreover, the 15% to 26% Palmer amaranth survival rate to the labeled dicamba rate exhibited by five populations in the greenhouse documents that Palmer amaranth at those locations has evolved dicamba resistance. Another step to confirm resistance is documenting heritability of the resistance between generations. The history of Palmer amaranth escaping dicamba was suggested when research in three of the study fields in 2021 showed just 55% control with the labeled dicamba rate and 69% control with 2 times the labeled rate. This suggests that dicamba resistance was passed down from the 2019 Palmer amaranth generation to the 2020 and 2021 generations. These findings are consistent with and reinforce other research in Tennessee that has documented dicamba resistance (1.85 to 14.25 relative resistance ratio) in Palmer amaranth (Foster and Steckel 2022).

The addition of malathion did not reverse dicamba resistance from populations collected from Tennessee. This suggests that metabolism-based resistance is not the methcanism for the poor dicamba performance. However, this does not completely rule out metabolic resistance or cytochrome P450s being key players in dicamba resistance due to the hundreds of P450 enzymes and other metabolic enzymes present in plants (Bak et al. 2011; Xu et al. 2015). This result was not consistent with results from the Macon County, GA, and Worth County, GA, locations, where malathion did improve control of large (15 to 38 cm) Palmer amaranth.

Future research should determine how well established the dicamba-resistant biotype of Palmer amaranth has become in Tenneessee and if these populations are cross-resistant to 2,4-D. Moreover, more detailed analysis of the relative dicamba resistance ratio of these populations should be conducted. In addition, research designed to assess the mechanism or mechanisms of resistance should be conducted on these biotypes. Finally, weed management research is warranted to determine how best to integrate herbicides and nonherbicide tactics to better control these Palmer amaranth populations.

Practial Implications

This research confirms that dicamba-resistant Palmer amaranth has evolved in Tennessee. It also suggests that cytochrome P450 metbolic resistance may not be the mechanism of resistance; therefore a farmer could not simply mix an organophosphate insecticide to improve Palmer amarnth control with dicamba. The decreased control of Palmer amarnth in Georgia may be an early indication of dicamba resistance evolving there.

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