

Interaction of Glufosinate with 2,4-D, Dicamba, and Tembotrione on Glyphosate-resistant *Amaranthus palmeri*

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21 **ABSTRACT**

Aims: Glyphosate-resistant *Amaranthus palmeri* S. Wats. (Palmer amaranth) is a major threat to crops in the southern USA. Experiments were conducted to evaluate differential tolerance to 2,4-D, dicamba, and tembotrione and interaction with glufosinate for GR-A. *palmeri* control.

Study Design: The differential tolerance experiment was conducted in a RCBD (randomized complete block design) with a split-plot arrangement of treatments with herbicide as mainplot and *A. palmeri* population as subplot. The herbicide interaction experiment was conducted in RCBD with factorial arrangement of treatments.

Place and Duration of Study: University of Arkansas-Fayetteville, USA. Tolerance experiments were conducted in October–November 2010 and in July–August 2011. Herbicide interaction experiments were conducted in the greenhouse in June–July 2011 and August–September 2011, and in the field in April–August 2012.

Methodology: Differential tolerance to alternative herbicides was evaluated using 12 GR-A. *palmeri* populations. Herbicide treatments were 0.047 and 0.094 kg ai/ha tembotrione, 1.06 and 2.12 kg ae/ha 2,4-D, and 0.56 and 1.12 kg ae/ha dicamba. In the herbicide interaction study, glufosinate at 0.18, 0.36, and 0.73 kg ai/ha were mixed with either 0.024, 0.047, and 0.094 kg ai/ha tembotrione; 0.28, 0.56, and 1.12 kg ae/ha 2,4-D; or 0.28 and 0.56 kg ae/ha dicamba. The population used was PRA-C.

Results: In the tolerance experiment, 2,4-D at 1x (1.06 kg ae/ha) practically killed all populations. Dicamba at 1x (0.56 kg ae/ha) killed an average of 36–94% of all plants per population, with survivors sustaining 80–99% injury. Tembotrione at 1x (0.094 kg ai/ha) caused 49–98% average mortality per population with survivors incurring 80–99% injury. In the herbicide interaction experiment, mixing half doses of glufosinate and 2,4-D resulted in 100% control of PRA-C in the greenhouse and field. At 1x, glufosinate controlled PRA-C 91% in the field; the addition of 0.5x or 1x of 2,4-D or 1x of dicamba resulted in 100% control.

Conclusion: In the field, 2,4-D was most effective on GR-A. *palmeri*, relative to dicamba and tembotrione. Some populations have a high risk of being selected for resistance to dicamba, glufosinate, and tembotrione. Glufosinate + 2,4-D mixtures were additive and at sublethal doses, synergistic. Most combinations of glufosinate and tembotrione were antagonistic.

Keywords: Antagonism; differential tolerance; herbicide mixtures; resistance; resistance management; selection; synergy

27 **1. INTRODUCTION**

Of all the *Amaranthus* species in the southern USA, *Amaranthus palmeri* L. (Palmer amaranth) is the most prolific, competitive, and common weed in crop fields[1,2,3,4]. The evolution of resistance to commonly used herbicides, like glyphosate, makes *A. palmeri* an economic concern for both crop growers and researchers. Apart from reducing crop yields[5,6,7,8], *A. palmeri* also interferes with production and processing activities such as harvesting and ginning cotton (*Gossypium hirsutum* L.)[9]. Historically, *A. palmeri* has been controlled with various herbicides with different modes of action. However, since the advent

of GR (glyphosate-resistant) crop technology, glyphosate has been applied alone or in mixture in corn (*Zea mays* L.) and soybean (*Glycine max* L.) production more than any other herbicide[10]. Most farmers use primarily glyphosate for weed control in GR crops because glyphosate has the broadest spectrum of weed control, no crop rotation restrictions, and low environmental and human risks[11]. Thus, resistance to glyphosate has evolved in an increasing number of weed species, including *A. palmeri*. The rapid increase in glyphosate-resistant weed populations[12] prompted the search for alternative weed management systems. Thus, Liberty Link® crop technology-based systems have come to the fore, along with development of crops with resistance to herbicides in other mode-of-action groups.

Herbicide-resistant populations arise from selection of a small group of resistant plants from the existing large population[13]. Such is the consequence of continuous application of the same herbicide or different herbicides with the same mode of action. Among the best practices recommended for resistance management is the use of herbicides with multiple modes of action in every season, with the use of soil-active herbicides being the foundation of a good chemical weed management strategy[12]. Even with the most active herbicides, it is not sustainable to always use the most economical weed control if that entails continuously using one mode of action. Resistance is promoted also by the use of herbicides with long residual activity or application of herbicides with a highly specific site of action[12,14,15]. Thus, integration of different modes of action, timing of application (soil and foliar) and interrupting weed exposure to the same mode of action is necessary to reduce or delay evolution of resistance. Toward this goal, crops resistant to 2,4-D, dicamba, HPPD (hydroxyphenylpyruvate dioxygenase) inhibitors, and possibly to the PPO (protoporphyrinogen oxidase) inhibiting herbicides are expected to be commercialized in the near future[16,17]. Scientists and crop producers look forward to having additional tools for weed management. On the other hand, widespread adoption of this new generation of herbicide-resistant crops and the subsequent increased use of dicamba, 2,4-D, tembotrione, and glufosinate are expected if the speed of adoption of GR technology is to be the norm. Eventually, advancements in herbicide-resistant crop technology and increased use of LibertyLink® technology will impact GR weed management strategies.

It is common knowledge that weed populations exhibit differential response to herbicides. For example, *A. palmeri* and *A. tuberculatus* populations respond differently to glyphosate, fomesafen, atrazine, and imazethapyr[18,19]. Variability in weed population response to herbicides has also been reported in other species such as *Oryza sativa* L. (weedy rice) [20]. Thus, testing for differential tolerance to alternative herbicides at the outset is informative in planning long-term weed management strategies.

One management strategy for delaying the selection of tolerant individuals from differentially tolerant populations is tank mixing herbicides. In addition to delaying or reducing the probability of resistance evolution in weed populations, tank mixing herbicides broadens the weed control spectrum and extends the effective weed control period if residual herbicides are included in the mixture[12,21,22,23]. Occasionally, some mixtures are synergistic while others are antagonistic^[21]. Both increased and reduced activities in tank mixtures involving glufosinate, dicamba, or mesotrione with other herbicides have been reported[25,26,27]. Not much is documented on the efficacy, or the interaction, of tank mixing glufosinate with tembotrione, dicamba or 2,4-D on *A. palmeri* control. Studies were conducted, firstly, to determine the response of *A. palmeri* populations to tembotrione, 2,4-D and dicamba, and secondly, to determine if glufosinate tank-mixed with the aforementioned herbicides can improve control of glyphosate-resistant *A. palmeri* while also evaluating the potential interaction between the mixtures.

2. MATERIAL AND METHODS

2.1 A. palmeri Response to Alternative Herbicides

Greenhouse experiments were conducted between October 2010 and July 2011 at the Altheimer greenhouses, Fayetteville, Arkansas, USA to evaluate the response of 12 *A. palmeri* populations to 2,4-D, dicamba, and tembotrione. Seeds were collected from 10 to 20 plants surviving at least two 0.84 kg ae/ha glyphosate applications in the 2009 growing season from growers' fields in Mississippi, Lee, Lawrence, Phillips, Conway, St. Francis, Prairie, and Poinsett counties, Arkansas. For general testing of resistance to glyphosate, from each individually sampled plant, 100 offsprings were grown in individual cells in trays (Redwayfeed Garden and Pet supply, 290 Briceland Rd, Reedway, CA 95560 USA) filled with Sunshine premix soil (Sunshine premix #1[®], Sun Gro Horticulture, 15831 NE 8th Street, Suite 100, Bellevue, WA 98008 USA). The trays were placed in the greenhouse with 34/26(±4) °C day/night temperatures. At the two-leaf stage, seedlings were thinned to one plant per cell and sprayed with 840 g ae/ha glyphosate at three-to-four-leaf stage. The herbicide was applied using a motorized boom equipped with two nozzles (TeeJet[®] 800067 flat-fan spray nozzles, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60189 USA) spaced 51 cm apart and calibrated to deliver 187 L/ha at 262 kPa. At 21 DAT (days after treatment) mortality data (number of dead plants) was recorded and visible injury of survivors were evaluated, using a scale of 0 (no injury) to 100% (complete death). Plants that were desiccated and showing no signs of regrowth at 21 DAT were considered dead. The assay was repeated. These populations were also tested for differential response to glufosinate (Bayer CropScience, 26 Davis Drive, Research Triangle Park, NC 27709, USA) at 0.73 kg ai/ha, using the same approach.

To test the differential response of populations to other alternative herbicides, 500 mg of seeds from each plant were mixed to make a composite subsample. Seeds from the composite were planted in cellular trays using the same procedure as in the glyphosate assay. When seedlings were about 7-cm tall, 2,4-D (Weedar 64[®], Nufarm Inc., 150 Harvester Drive, Burr Ridge, IL, 60527, USA), dicamba (Clarity[®], BASF Corp., P.O. Box 13528, Research Triangle Park, NC 27709, USA), or tembotrione (Laudis[®], Bayer CropScience, 26 Davis Drive, Research Triangle Park, NC 27709, USA), were applied at 1x and 2x of 1.06 and 0.56 kg ae/ha for 2,4-D and dicamba, respectively, and 0.5x and 1x of 0.094 kg ai/ha for tembotrione. The 1x dose of tembotrione was based on the Laudis[®] label for corn for *Amaranthus* <15 cm tall. We tested only up to 1x dose of tembotrione because we expected that *A. palmeri* would be highly sensitive in the greenhouse and that population differentiation would be evident only at a sublethal dose. On the other hand, we expected that 2,4-D and dicamba would show enough population differentiation at the 1x dose and we wanted to test if any tolerant individuals could survive a 2x dose of these herbicides. AMS (ammonium sulfate) at 10 g/L and MSO (methylated seed oil) at 1% v/v were added to tembotrione. The treated seedlings were returned to the greenhouse and maintained for 21 days when the evaluation was conducted.

A. palmeri response to alternative herbicides was evaluated in separate experiments. The experimental design was RCBD (randomized complete block design) with a split-plot treatment arrangement of herbicide doses (mainplot) and populations (subplot). The herbicide dose and population combination were replicated four times. In each replicate, 25 plants per population were evaluated, for a total of 100 plants per run. The experiment was repeated. Treated plants were assessed individually for visual injury 21 DAT on a scale of 0 (no injury) to 100% (complete death) relative to the nontreated plants. Analysis of variance on % injury and % mortality data for each experiment was performed using PROC GLM and PROC GLIMMIX, respectively, in SAS (SAS[®], v9.2, SAS Institute, P.O. Box 8000, Cary, NC 25712-8000). Fisher's Protected LSD (Least Significant Difference) was used to separate

rate, population, and interaction means, where necessary, at $P = 0.05$. Frequency analysis was also done, where necessary, to evaluate tolerance distribution of individual plants of the test populations to the labeled doses of herbicides.

2.2 Herbicide Interaction Assay

Herbicide interaction experiments were conducted both in the greenhouse and field. Greenhouse experiments were conducted between June and August 2011 at the Altheimer greenhouses, Fayetteville, Arkansas, USA. For this experiment, Pra-C population was used because it showed 30% survival frequency to the labeled dose of glufosinate in previous assays. Pra-C was obtained from a field planted with GR-cotton for more than 10 years. *A. palmeri* seeds were planted and managed as in the herbicide response assay in Section 2.1. The doses of glufosinate (Factor A) were 0.25x, 0.5x, and 1x of 0.73 kg ai/ha. The tank-mix options (Factor B) were tembotrione at 0.25x, 0.5x, or 1x (0.094 kg ai/ha); 2,4-D at 0.25x, 0.5x or 1x (1.06 kg ae/ha), or dicamba at 0.5x or 1x (0.56 kg ae/ha). Ammonium sulfate was added at 10 and 20 g/L, respectively, to tembotrione-alone and glufosinate-alone treatments. Methylated seed oil was also added to all tembotrione treatments at 1% v/v. All treatments were applied at 276 kPa in 187 L/ha water. After herbicide application, the dicamba- and 2,4-D-treated plants were placed in separate greenhouses for 24 h to avoid herbicide vapor drift to other treatments; thereafter, all plants were kept in one greenhouse. The plants were watered as needed and fertilized weekly (Miracle-Gro® all-purpose plant food containing 15-30-15% NPK, The Scotts Co. LLC, Marysville, OH, USA) for an additional 21-day period when herbicide efficacy was evaluated.

The field experiment was conducted in 2012 at the Vegetable Research Station, Kibler, Arkansas. Experimental plots were 2.4 m², with 0.6- and 1.2-m alleys between plots and replicates, respectively. *A. palmeri* was hand-seeded 20 cm apart in the middle 1.2 m² leaving an additional 0.6-m border around the plot, resulting in a density of 25 plants m⁻². The herbicide treatments were applied when seedlings were 6 – 8 cm tall. The same doses and tank mixtures as in the greenhouse experiment were used. Herbicides were sprayed using a CO₂ backpack sprayer with 3-nozzle (TeeJet® 11002 VS, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60189 USA) spaced 51 cm apart and calibrated to deliver 187 L/ha at 248 kPa. The mean relative humidity (RH) for the greenhouse and field experiments was 52 and 32%, respectively.

Both greenhouse and field experiments were laid out in RCBD and treatments were replicated four times. The experiments were conducted twice in the greenhouse and once in the field. *A. palmeri* seedlings were visually rated for overall control at 21 DAT on a scale of 0 to 100% for no visible effect and total death, respectively. The herbicide interaction was evaluated using the equation described by Colby[28] shown below;

$$E_1 = (X_1 + Y_1) - (X_1 Y_1 / 100) \quad (1).$$

Where E_1 is the expected efficacy from the combination of herbicides A and B; X_1 and Y_1 are the observed efficacy from herbicide A and B alone, respectively. The expected efficacy of the tank mixture was calculated using observed control values from individual herbicides used in the mixture within each replicate. The expected efficacy (E_1) was then compared with the observed efficacy obtained from the tank mix using Fisher's Protected LSD[30,31]. The interaction was considered to be synergistic if the observed plant response to the herbicide mixture was significantly higher than the expected, or antagonistic if the observed response was significantly lower than the expected, or additive if the observed and the expected responses were similar. Data were subjected to ANOVA using PROC GLM in SAS and means were separated by Fisher's protected LSD at the 5% level of significance.

3. RESULTS AND DISCUSSION

3.1 Response to Herbicides in the Greenhouse

All 12 *A. palmeri* populations used in this research had some GR individuals ranging from 14 - 9% (Table 1). Pra-C population had the highest frequency of GR plants. The Pra-C field had been in continuous GR-cotton for more than 10 years. Follow-up bioassays of these populations with glufosinate showed a few individuals escaping the 1x dose, but were highly injured to produce seed (data not shown). An exception was Pra-C where an average of 30% of individuals survived the full dose of glufosinate (Table 1). As expected, intrapopulation variation was manifested at sublethal doses.

3.1.1 Response to Tembotrione in Greenhouse Assays

No interaction involving population and tembotrione dose was observed. The run effect was not significant; therefore, data were averaged over runs. Differential response to tembotrione among *A. palmeri* populations was evident especially at the sublethal dose. The full dose of tembotrione killed an average of 49 - 99% of plants in each population (Table 2) and the survivors incurred 80 - 99% injury, 21 DAT (Table 1). For sensitive *Amaranthus tuberculatus* (waterhemp), tembotrione controlled 91 - 96% of the population in the greenhouse[32]. Among the Arkansas populations, at the 1x dose, STF-B and STF-C showed the lowest injury followed by Mis-B, Mis-C. The survival rate of plants at the 1x dose of tembotrione ranged from 2% in Lee-A to 51% in Mis-C populations (Table 2). Plants tolerant to the 1x dose were more frequent in STF-B, STF-C, and Mis-B where 45, 26, and 23%, respectively, of the survivors had <50% injury (Fig. 1). Law-B, Law-C, Con-A, Cri-C, Phi-B, Pra-C, and STF-A had less than 10% of survivors with ≤50% injury indicating that, if such plants were in the field under reduced competition from other weeds and the crop, some of these survivors could produce seed. On the other hand, all survivors in Lee-A and Cri-C had ≥50% injury and were not likely to reproduce. Populations STF-B, STF-C, and Mis-B would need additional control strategy to prevent seed production. The most tolerant plants had new growth with no injury symptoms 14 DAT. Having 18 - 27% of Mis-B, STF-C, and STF-B survivors with less than 50% injury when treated with the labeled dose suggests that these populations have higher risk than others for resistance selection by tembotrione.

Variation in population response to tembotrione was also observed between HPPD-susceptible- and -resistant ecotypes. An experiment by Lally[33], in the greenhouse, showed 100% control of susceptible *A. palmeri* with 0.094 kg ai ha⁻¹ tembotrione, but only 66 - 82% control of HPPD-resistant populations. Plant size and environmental differences during seedling development and around the time of herbicide application are critical determinants of herbicide efficacy. In the field, Lally[33] observed even lower activity of tembotrione on *A. palmeri* (40 - 63% control) primarily because the plants were 15-27 cm tall at application time; the label recommends spraying the herbicide when *Amaranthus* species are <6-cm tall[34]. When sprayed at the correct size, 90 - 100% control of *A. palmeri* was achieved with 0.094 kg ha⁻¹ tembotrione[35].

Table 1. Response of 12 *Amaranthus palmeri* populations 21 days after treatment (DAT) with tembotrione, 2,4-D, and dicamba in greenhouse experiments.

Population	Frequency of glyphosate-resistant plants (%) ^a	Mortality at the 1x dose of glufosinate (%) ^b	<i>Amaranthus palmeri</i> response (% injury) ^{c,d}					
			tembotrione		2,4-D		dicamba	
			0.5x	1x	1x	2x	1x	2x

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Con-A	95	94	74	95	99	100	97	99
Cri-C	36	92	72	97	99	100	99	99
Law-B	16	93	69	94	100	100	99	99
Law-C	14	85	84	97	99	100	99	99
Lee-A	88	100	82	99	100	100	99	99
Mis-B	50	91	77	88	99	100	97	99
Mis-C	57	90	69	89	100	100	99	98
Phi-B	17	100	71	93	99	100	99	99
Pra-C	99	71	63	95	99	100	80	98
STF-A	83	100	60	94	100	100	85	99
STF-B	39	95	75	80	99	100	98	98
STF-C	90	88	71	84	99	100	98	99
LSD _{0.05} within herbicide ^e			7		- ^g		3	
Within herbicide and dose ^f		-	6		-		3	

226 ^aA total of 200 seedlings per population were sprayed with glyphosate, 0.84 kg ae/ha, at the
227 3-4 leaf stage.

228 ^bA total of 200 seedlings per population were sprayed with glufosinate, 0.73 kg ai/ha, at the
229 3-4 leaf stage.

230 ^cInjury based on visual rating on a scale of 0 (no effect) to 100% (total death) 21 DAT. Each
231 value is the average of 4 replicates, 25 plants/replicate, and two runs.

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232 ^dTembotrione in kg ai/ha; 2,4-D and dicamba in kg ae/ha; 1x doses for tembotrione, 2,4-D,
233 and dicamba are 0.094, 1.06, and 0.56 kg/ha, respectively.

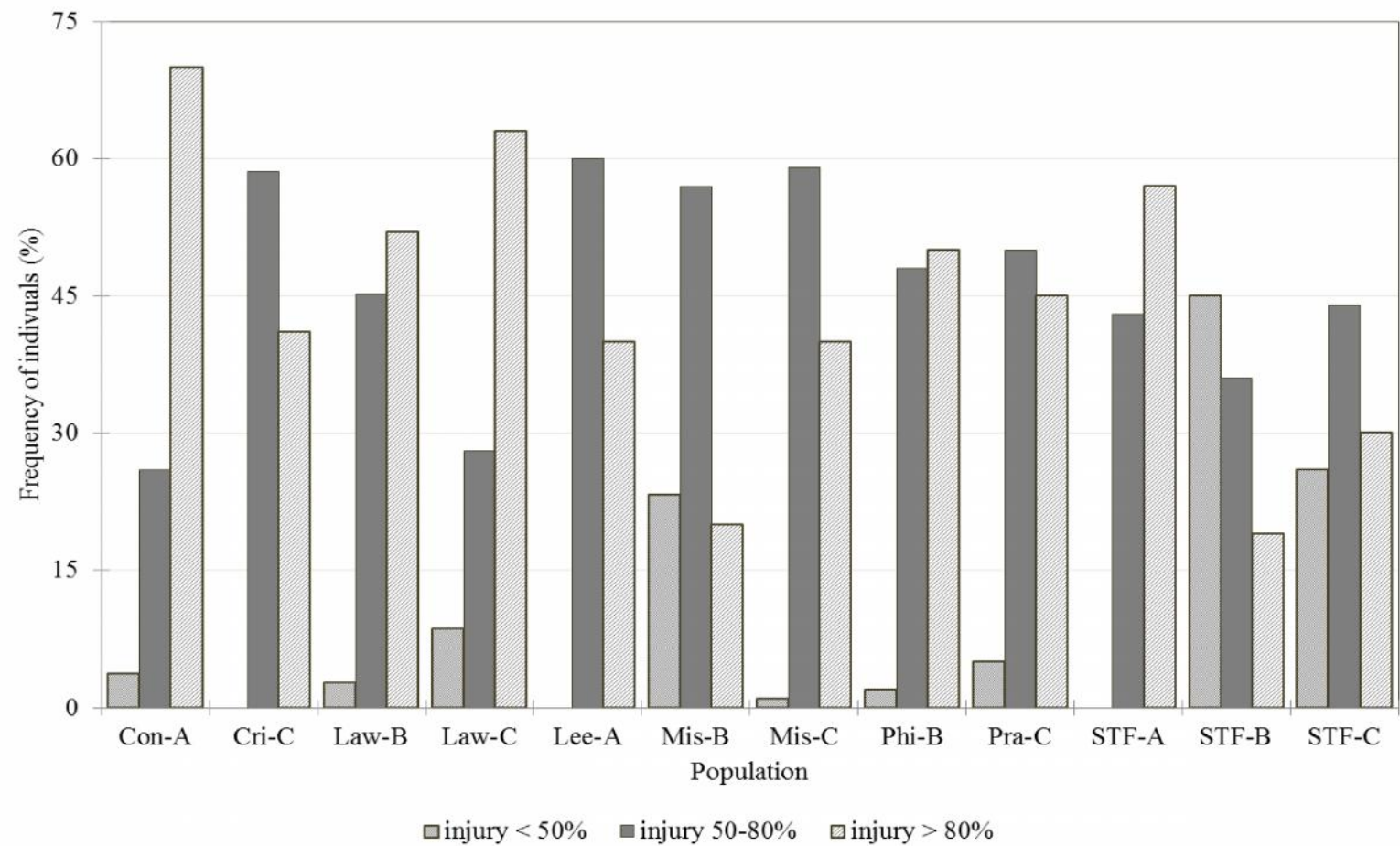
234 ^eFisher's Protected LSD to compare dose means at the same or different populations;

235 ^fLSD to compare population means within the same herbicide dose.

236 ^gData for 2,4-D were not subjected to ANOVA due to minimal or no variance among
237 replicates and runs.
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3.1.2 Response to 2,4-D in Greenhouse Assays

Due to minimal or no variance among replicates, the data were not subjected to ANOVA. All populations were highly sensitive to the 1x dose of 2,4-D, which killed 96-100% of plants 21 DAT and the majority of those surviving this application were 99% injured on average (Table 1 and 2). The few surviving plants had at least 80% injury at the time of evaluation, and were unlikely to reproduce. 2,4-D also showed >90% control of GR *Amaranthus tuberculatus* (tall waterhemp) in Mississippi[36], but less than 90% control was also reported on GR-A. *palmeri* and *Amaranthus spinosus* L. (spiny amaranth)[37,38]. In their experiments, 2,4-D controlled *A. palmeri* better than did dicamba or glufosinate, similar to the results of our experiments.

Table 2. Mortality of 12 *Amaranthus palmeri* populations 21 days after tembotrione, 2,4-D, and dicamba treatments in greenhouse experiments.

Population	<i>A. palmeri</i> mortality (%) ^{a,b}					
	tembotrione		2,4-D		dicamba	
	0.5x	1x	1x	2x	1x	2x
Con-A	38	73	98	100	89	97
Cri-C	32	86	98	100	89	95
Law-B	38	64	100	100	94	96
Law-C	47	82	99	100	88	94
Lee-A	46	98	100	100	91	95
Mis-B	43	70	97	100	73	92
Mis-C	34	49	100	100	73	93
Phi-B	28	67	99	99	81	94
Pra-C	41	79	96	100	36	74
STF-A	20	70	100	100	36	86
STF-B	44	61	98	100	80	93
STF-C	54	64	99	100	79	94
LSD _{0.05}						
Within herbicide ^c	7		1		7	
Within herbicide and dose ^d	14		- ^e		14	

^aPercent survival of plants treated with the herbicides 21 DAT. Plants were grown in cellular trays at 1 plant/cell and sprayed with 0.84 kg ae/ha glyphosate at 3-4 leaf stage. Each value is the average of 4 replicates, 25 plants/replicate, and 2 runs.

^bTembotrione in kg ai/ha; 2,4-D and dicamba in kg ae/ha. 1x doses for tembotrione, 2,4-D, and dicamba are 0.094, 1.06, and 0.56 kg/ha, respectively.

263 ^cFisher's protected LSD to compare dose means at the same or different populations.

264 ^dLSD to compare population means within the same herbicide dose.

265 ^eData for 2,4-D were not subjected to ANOVA due to minimal or no variance among
266 replicates and runs.

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269 **3.1.3 Response to Dicamba in Greenhouse Assays**

270 Dicamba dose by population interaction effect on *A. palmeri* injury was significant ($P < .001$),
271 but not on mortality ($P = .14$). The full dose of dicamba caused an average of 80 – 99%
272 injury on surviving plants per population, 21 DAT (Table 1), but some plants showed signs of
273 recovery. The 2x dose of dicamba did not kill all plants in the less sensitive populations. The
274 use of 2x rate, of course, is not recommended. Mortality ranged from 36 - 94% across
275 populations, with Pra-C and STF-A being the most tolerant, having at least 60% survivors, of
276 which 8 and 2%, respectively, had $\leq 50\%$ injury (Table 2; Fig. 2). On the other hand, 11% of
277 treated plants from Con-A survived 1x dicamba, 16% of which had $< 50\%$ injury.
278 Supplemental control measures are needed to prevent these survivors from producing
279 seeds. Different responses to dicamba have been reported by other researchers. Doherty *et*
280 *al.*[39] reported 99% control of 7.5- and 15-cm GR-A. *palmeri* by 0.58 kg ha⁻¹ dicamba while
281 Johnson[40] reported a wide range of dicamba activity (61-97%) on *A. palmeri*. One major
282 reason for the large variation in these results is differential tolerance between populations.
283 Populations with more inherently tolerant individuals have higher likelihood of evolving
284 resistance[14,15].

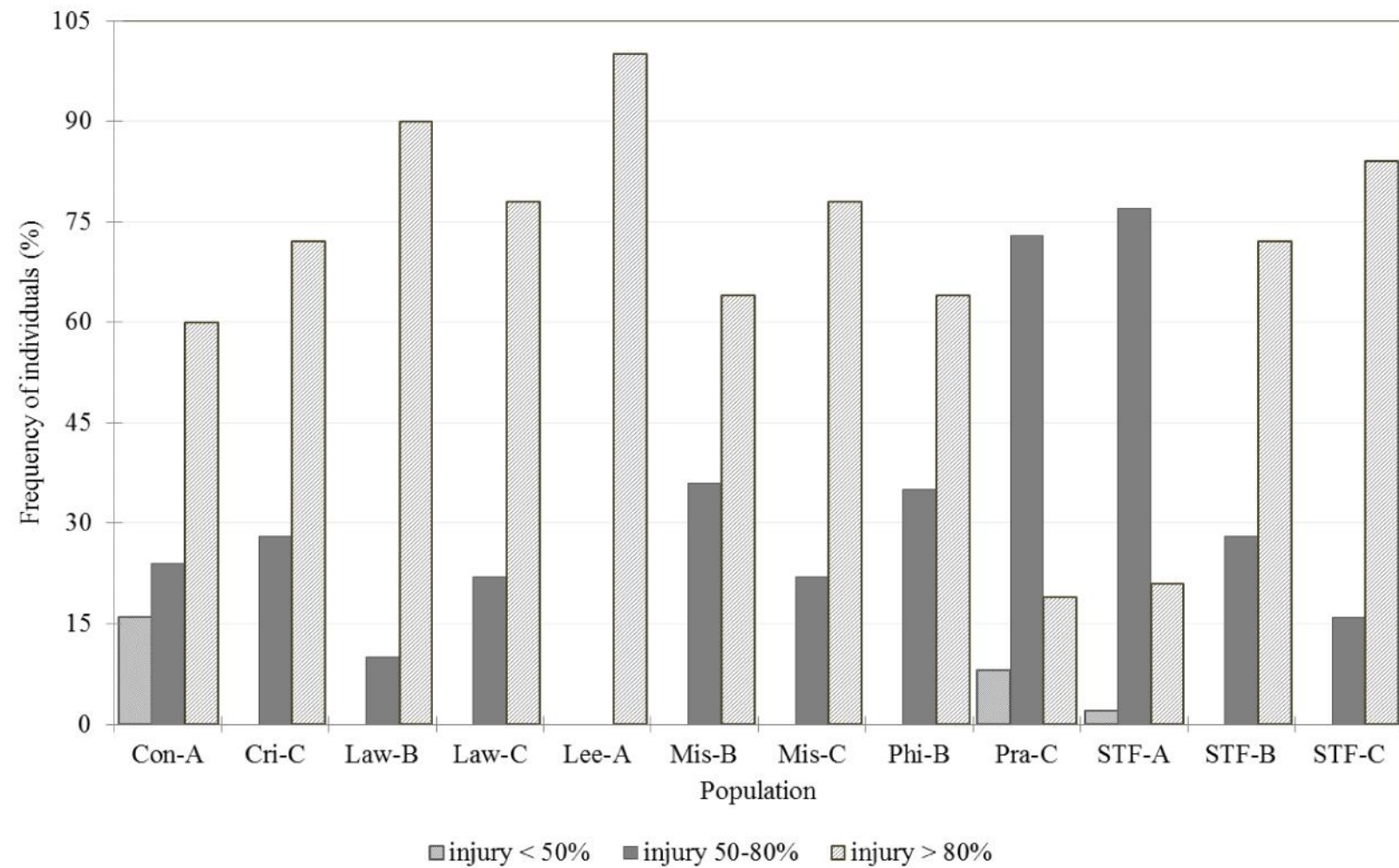
285 **3.2 Glufosinate Interaction with Alternative Herbicides**

286 When applied separately in the field, no difference between 0.5x and 1x doses of dicamba
287 on *A. palmeri* injury was observed, but full doses of tembotrione, 2,4-D, and glufosinate all
288 performed better than their 0.5x counterparts (Table 3). The 1x doses of glufosinate and
289 2,4-D had similar activity on *A. palmeri* (91 and 93%, respectively) and was better than the
290 1x doses of dicamba (83%) and tembotrione (69%). Although tembotrione applied at the 1x
291 dose was as effective as glufosinate, 2,4-D, and dicamba in the greenhouse (Tables 1 and
292 2), it provided the least control of *A. palmeri* in the field.

293 Generally, the efficacy of 1x doses of glufosinate, dicamba, and tembotrione was lower in
294 the field than in the greenhouse. Unlike plants in the field, plants in the greenhouse are
295 exposed to less solar radiation and are not exposed to physical or environmental stresses.
296 Greenhouse-grown plants are not hardened; therefore, are more susceptible to herbicides.
297 The field environment is associated with heat stress, low RH and sometimes low soil
298 moisture content, with intense solar radiation. Physiologically, these are preconditions for the
299 synthesis of thicker leaf cuticles and lignification of structural organs, which reduce herbicide
300 absorption[41,42,43,44]. Anderson *et al.*[45] reported a reduction from 100% control of
301 *Setaria viridis* L. (green foxtail) at 95% RH to 30% growth inhibition at 40% RH. The mean
302 RH for our greenhouse and field experiments was 52 and 32%, respectively. At higher RH,
303 herbicide spray droplets evaporate slower; meanwhile the leaf cells are turgid and stomates
304 are open (granting that plants are not water-stressed), overall allowing maximum absorption
305 of herbicide. Thus, higher RH favors good activity of foliar herbicides.

306

Figure 2



310 **Table 3. Efficacy and compatibility of glufosinate tank-mixed with tembotrione, 2,4-D**
311 **or dicamba on *Amaranthus palmeri* under field conditions, Kibler, Arkansas, USA**
312 **2012.**

Tank-mix option	Dose ^a	<i>A. palmeri</i> control (%) ^b			
		glufosinate dose (kg ai/ha)			
		0 ^c	0.25x	0.5x	1x
None	0.00	0.0	38	84	91
Tembotrione	0.25x	18	53	56 (87)-	60 (93)-
Tembotrione	0.5x	59	56 (87)- ^d	73 (94)-	78 (97)-
Tembotrione	1x	69	60 (93)-	93 (97)-	92 (99)-
2,4-D	0.25x	43	80 (65)+	88	94
2,4-D	0.5x	76	96 (84)+	100	100
2,4-D	1x	93	92	100	100
Dicamba	0.5x	83	67 (89)-	81 (94)-	94
Dicamba	1x	83	76 (88)-	93	100
^c LSD _{0.05}		----- 3 -----			

313 ^aTembotrione 1x dose = 0.094 kg ai/ha; 2,4-D 1x dose = 1.06 kg ae/ha; dicamba 1x dose =
314 0.56 kg ae/ha; glufosinate 1x dose = 0.73 kg ai/ha.

315 ^bVisual rating 21 days after treatment on a scale of 0 to 100%; 0 = no visible response, 100
316 = total death. Each value is the average of 4 replicates, 25 plants/replicate.

317 ^cFisher's Protected LSD to compare treatment means within the same columns or across
318 columns.

319 ^dValues in parentheses, calculated by Colby (1967) method using the raw data, indicate the
320 expected activity from tank mixtures having significant differences between the observed
321 and the expected at $P = 0.05$. A negative sign (-) denotes an antagonistic interaction; a
322 positive (+) sign denotes synergistic interaction.

323 324 **3.2.1 Glufosinate interaction with 2,4-D**

325 **In the field and greenhouse**, the data showed favorable interaction between glufosinate and
326 2,4-D for the control of *A. palmeri* population PRA-C (Tables 3 and 4). **In the field,**
327 **glufosinate alone controlled PRA-C 91%**; the addition of 0.25x 2,4-D improved weed control
328 to 94% while the addition of 0.5x 2,4-D attained 100% control (Table 3). Interestingly, mixing
329 half doses of 2,4-D and glufosinate resulted in 100% control, demonstrating additive effects
330 according to Colby's test[28]. Synergistic interaction was observed when 0.25x dose of
331 glufosinate (0.18 kg ai/ha) was mixed with 0.25x or 0.5x dose of 2,4-D. The synergistic
332 mixture of 0.25x glufosinate with 0.5x 2,4-D resulted in 96% control of *A. palmeri*, which is
333 already excellent for commercial crop production although not favorable for long-term
334 resistance management. The goal should be 100% control[12] and in this research, this was

achieved with the mixture of 0.5x or 1x doses of glufosinate and 2,4-D. In the greenhouse, the 1x dose of glufosinate or 2,4-D alone controlled the plants 99-100% (Table 4). Combinations of any dose of 2,4-D with at least 0.5x dose of glufosinate resulted in 100% control of *A. palmeri* in the greenhouse.

Additive or synergistic interactions were also observed with tank mixtures of glyphosate at sublethal dose (0.28 kg ae/ha) and 2,4-D on *Convolvulus arvensis* L. (field bindweed)[29]. Tank mixes of reduced doses that result in 100% weed control are economical and environmentally friendly, but they should be tested in a wide array of environments and geographies for robustness of performance. Otherwise, if the mixture of reduced rates fails to attain 100% control, then selection for resistant plants ensues. Such mixture should also be used in conjunction with all other weed management practices.

3.2.2 Glufosinate Interaction with Dicamba

Antagonism was observed both in the field and greenhouse when <1x glufosinate dose was mixed with 0.5x dose of dicamba (Tables 3 and 4) or, when 0.25x glufosinate was mixed with 1x dose of dicamba in the field (Table 3). The full dose of dicamba provided additional control of *A. palmeri* when mixed with either 0.5x or 1x glufosinate. With these herbicides, 100% control of *A. palmeri* in the field was achieved only with the mixture of full doses. Thus, dicamba is also a good supplemental herbicide to use in Liberty Link® crop production system. The efficacy of herbicide combinations need to be tested because interactions of different chemistries differ and these are further modified by dose and species. In contrast to the glufosinate-dicamba interaction effect on *A. palmeri*, mixtures of dicamba with sublethal dose of glyphosate resulted in additive or synergistic control of *C. arvensis*[29].

3.2.3 Glufosinate Interaction with Tembotrione

The mixture of 1x doses of glufosinate with 0.25 or 0.5x dose of tembotrione was antagonistic, resulting in a reduction of *A. palmeri* control by 4 to 7% points both in the field (Table 3) and the greenhouse (Table 4). Antagonism was observed in about 90% of the glufosinate + tembotrione mixtures in both experiments. The full dose of tembotrione did not add to the activity of the full dose of glufosinate, although the addition of tembotrione would still be beneficial in improving control of other weed species less sensitive to glufosinate such as annual grasses. Synergistic or antagonistic interaction of glufosinate with other herbicides in the tank mixture has been reported previously. Burke *et al.*[46] observed that the activity of clethodim applied at 105 or 140 g/ha on *Eleusine indica* L. (goosegrass) was antagonized by at least 52% when mixed with 290 or 410 g ai/ha glufosinate. Antagonism was also noted on *Sorghum halepense* L. (johnsongrass), *Brachiaria platyphylla* L. (broadleaf signalgrass), *Panicum dichotomiflorum* Michx. (fall panicum), *E. indica*, and *Digitaria sanguinalis* (L.) Scop. (large crabgrass), when 468 g ai/ha glufosinate was mixed with clethodim, fluazifop-P, quizalofop-P, or sethoxydim[47]. In practice, some level of antagonism is acceptable as long as the overall weed control remains excellent and the desired expansion of weed control spectrum, or maximum control of a herbicide-resistant species, is achieved. Conversely, a synergistic tank mixture is useful only if the improvement in herbicide activity makes such mixture one of the best options available. Tank mixtures for the control of a highly prolific weed, such as *A. palmeri*, need to be 100% effective to prevent proliferation of resistant plants.

Table 4. Efficacy and compatibility of glufosinate tank-mixed with tembotrione, 2,4-D or dicamba on *Amaranthus palmeri* in the greenhouse, Fayetteville, Arkansas, USA 2011.

Tank-mix option	Dose ^a	<i>A. palmeri</i> control (%) ^b			
		glufosinate dose			
		0	0.25x	0.5x	1x
None		0.0	70	94	99
Tembotrione	0.25x	64	66 (91)- ^d	78 (99)-	92 (100)-
Tembotrione	0.5x	93	90 (98)-	97 (100)-	94 (100)-
Tembotrione	1x	98	97 (100)-	100 (100)	100
2,4-D	0.25x	88	99	100	100
2,4-D	0.5x	97	99	100	100
2,4-D	1x	100	100	100	100
Dicamba	0.5x	93	95 (98)-	96 (100)-	100
Dicamba	1x	100	100	100	100
^c LSD _{0.05}		----- 6 -----			

387 ^aTembotrione 1x dose = 0.094 kg ai/ha; 2,4-D 1x dose = 1.06 kg ae/ha; dicamba 1x dose =
388 0.56 kg ae/ha; glufosinate 1x dose = 0.73 kg ai/ha.

389 ^bVisual rating 21 days after treatment on a scale of 0 to 100%; 0 = no visible response, 100
390 = total death; n = 200.

391 ^cFisher's protected LSD to compare treatment means within the same or across columns.

392 ^dValues in parentheses, calculated by Colby (1967) method using the raw data, indicate the
393 expected activity from tank mixtures having significant differences between the observed
394 and the expected at $P = 0.05$. A negative sign (-) denotes an antagonistic and a positive (+)
395 denotes synergistic response.

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399 4. CONCLUSION

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401 Glyphosate-resistant *A. palmeri* populations respond differentially to tembotrione, 2,4-D, and
402 dicamba. Pra-C population has higher tolerance to both 2,4-D and dicamba than the rest.
403 Generally, only a small percentage of GR-*A. palmeri* can survive the full dose of 2,4-D or
404 dicamba. Nevertheless, the survivors should be prevented from producing seed, using
405 supplemental weed control practices. Otherwise, we predict that selection for resistance to
406 dicamba will occur quickly. Similarly, higher dose of tembotrione, sequential application, or
407 compatible tank mixtures are needed to maximize *A. palmeri* control. The majority of
408 glufosinate + tembotrione and some glufosinate + dicamba tank mixtures were antagonistic
409 in the field. Among the alternative herbicides, tembotrione was the least beneficial tank-mix
410 partner with glufosinate for controlling GR-*A. palmeri* in these experiments. Glufosinate and

2,4-D tank mixtures were generally additive and at reduced doses, synergistic. The half rates of 2,4-D and glufosinate can be tank-mixed to achieve 100% *A. palmeri* control. The robustness of this treatment needs to be tested across a wide range of environments.

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COMPETING INTERESTS

The authors have no competing interests.

AUTHORS' CONTRIBUTIONS

Author GMB, conducted the experiment, collected and analyzed data, prepared the first draft of the manuscript and worked with NRB in revising the manuscript. NRB collected the plant samples from farmers' fields together with EAA, designed the experiment, led the manuscript revision, and finalized the manuscript. EEG guided the data analysis and interpretation. EAA and RAS helped in the experiment implementation and data collection. RCS contributed to the finalization of treatments and guided the practical interpretation of results. All authors approved the manuscript.

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569

570 **FIGURE LEGENDS**

571 Figure 1. Injury frequency distribution of *A. palmeri* plants surviving 0.094 kg ai/ha
572 tembotrione in greenhouse experiments. Tembotrione was applied, to 7-cm-tall seedlings,
573 with 10 g/L ammonium sulfate and 1% by volume methylated seed oil, in 187 L/ha spray
574 volume. Visual rating was conducted 21 days after treatment. The data set was comprised of
575 individual ratings of 200 plants per population (4 replications, 25 plants per replication, 2
576 runs).

577 Figure 2. Injury frequency distribution of *A. palmeri* plants surviving 0.56 kg ae/ha dicamba in
578 greenhouse experiments. Visual rating was conducted 21 days after treatment. Dicamba
579 was applied to 7-cm-tall seedlings in 187 L/ha spray volume. Visual rating was conducted 21
580 days after treatment. The data set was comprised of individual ratings of 200 plants per
581 population (4 replications, 25 plants per replication, 2 runs).

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