

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

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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 glyphosate-resistant (GR) *A. palmeri* control.

Study Design: The differential tolerance experiment was conducted in a randomized complete block design (RCBD) 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 1.06 kg ae/ha 2,4-D, 0.56 kg ae/ha dicamba, and 0.094 kg ai/ha tembotrione. 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) killed 96–100% of plants per population. Dicamba at 1x (0.56 kg ae/ha) killed 36–94% of plants per population, with survivors sustaining 80–99% injury. Tembotrione at 1x (0.094 kg ai/ha) caused 49–98% 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

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 producers 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 having different modes of action. However, since the advent of glyphosate-resistant (GR) 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 glyphosate primarily 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[10]. Thus, resistance to glyphosate has evolved in an increasing number of weed species, including *A. palmeri*. The rapid increase in GR weed populations[11] 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 (HR) biotypes arise from individual plants within a population. By the time groups of HR plants are noticed, the evolutionary process is well advanced[12]. 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[11]. 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 also promoted by the use of herbicides with long residual activity or application of herbicides with a highly specific site of action[11,13,14]. Thus, integration of herbicides with 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[15,16]. Scientists and crop producers look forward to having additional tools for weed management. On the other hand, widespread adoption of this new generation of HR 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 HR crop technology will impact HR 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[17,18]. Variability in weed population response to herbicides has also been reported in other species such as *Oryza sativa* L. (weedy rice) [19]. 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[11,20,21,22]. Occasionally, some mixtures are synergistic while others are antagonistic[23]. Both increased and reduced activities in tank mixtures involving glufosinate, dicamba, or mesotrione with other herbicides have been reported[23,24,25]. 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 GR *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 GR *A. palmeri* while also evaluating the potential interaction between the mixtures.

2. MATERIAL AND METHODS

2.1 Response to Alternative Herbicides in the Greenhouse

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 per population, surviving at least two 0.84 kg ae/ha glyphosate applications in the 2009 growing season from producer fields in Mississippi, Lee, Lawrence, Phillips, Conway, St. Francis, Prairie, and Poinsett counties in Arkansas. Acid equivalent (ae) was used as the unit of measure for glyphosate, 2,4-D and dicamba because these herbicides are formulated as different salts of the acid form of the herbicidal molecule. Glyphosate, for example, is formulated as salts of isopropylamine, trimethyl sulfonium, or potassium. To make herbicide doses comparable across different formulations, the standard protocol is to calculate the dose of this type of herbicides based on the acid concentration. 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 days after treatment (DAT) 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) relative to the nontreated check. 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 per population. 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 doses of 1.06 and 0.56 kg ae/ha for 2,4-D and dicamba, respectively, and 0.094 kg ai/ha for tembotrione. The 1x dose of tembotrione was based on the Laudis[®] label for corn for

121 *Amaranthus* <15 cm tall. Ammonium sulfate (AMS) at 10 g/L and methylated seed oil (MSO)
122 at 1% v/v were added to tembotrione based on commercial recommendation. The other
123 herbicides do not need additives. The treated seedlings were returned to the greenhouse
124 and maintained for 21 days when the evaluation was conducted.

125 *A. palmeri* response to alternative herbicides was evaluated in separate experiments. The
126 experimental design was randomized complete block (RCBD) with a split-plot treatment
127 arrangement of herbicide dose (mainplot) and population (subplot). Treatments were
128 replicated four times. In each replicate, 25 plants per population were evaluated, for a total of
129 100 plants per run. The experiment was repeated. Treated plants were assessed individually
130 for visual injury 21 DAT on a scale of 0 (no injury) to 100% (complete death) relative to the
131 nontreated plants. Analysis of variance on % injury and % mortality data for each experiment
132 was performed using PROC GLM and PROC GLIMMIX, respectively, in SAS (SAS®, v9.2,
133 SAS Institute, P.O. Box 8000, Cary, NC 25712-8000). Fisher's protected least significant
134 difference (LSD) was used to separate means, where necessary, at $P=0.05$. Frequency
135 analysis was also done, where necessary, to evaluate tolerance distribution of individual
136 plants of the test populations to the labeled doses of herbicides.

137 **2.2 Glufosinate Interaction with Alternative Herbicides**

138 Herbicide interaction experiments were conducted both in the greenhouse and field.
139 Greenhouse experiments were conducted between June and August 2011 at the Alzheimer
140 Laboratory, Fayetteville, Arkansas, USA. For this experiment, Pra-C population was used
141 because it showed 30% survival frequency to the labeled dose of glufosinate in previous
142 assays. Pra-C was obtained from a field planted with GR-cotton for more than 10 years. *A.*
143 *palmeri* seeds were planted and managed as in the herbicide response assay in Section 2.1.
144 The doses of glufosinate (Factor A) were 0.25x, 0.5x, and 1x (0.73 kg ai/ha). The tank-mix
145 options (Factor B) were tembotrione at 0.25x, 0.5x, or 1x (0.094 kg ai/ha); 2,4-D at 0.25x,
146 0.5x or 1x (1.06 kg ae/ha), or dicamba at 0.5x or 1x (0.56 kg ae/ha). Ammonium sulfate was
147 added at 10 and 20 g/L, respectively, to tembotrione-alone and glufosinate-alone treatments.
148 Methylated seed oil was also added to all tembotrione treatments at 1% v/v. All treatments
149 were applied at 276 kPa in 187 L/ha water. After herbicide application, the dicamba- and 2,4-
150 D-treated plants were placed in separate greenhouses for 24 h to avoid herbicide vapor drift
151 to other treatments; thereafter, all plants were kept in one greenhouse. The plants were
152 watered as needed and fertilized weekly (Miracle-Gro® all-purpose plant food containing 15-
153 30-15% NPK, The Scotts Co. LLC, Marysville, OH, USA) for an additional 21-day period
154 when herbicide efficacy was evaluated.

155 The field experiment was conducted in 2012 at the Vegetable Research Station, Kibler,
156 Arkansas. Experimental plots were 2.4 m², with 0.6- and 1.2-m alleys between plots and
157 replicates, respectively. *A. palmeri* was hand-seeded 20 cm apart in the middle 1.2 m²
158 leaving an additional 0.6-m border around the plot, resulting in a density of 25 plants m⁻².
159 The herbicide treatments were applied when seedlings were 6 – 8 cm tall. The same doses
160 and tank mixtures as in the greenhouse experiment were used. Herbicides were sprayed
161 using a CO₂ backpack sprayer with 3-nozzles spaced 51 cm apart and calibrated to deliver
162 187 L/ha at 248 kPa. The mean relative humidity (RH) for the greenhouse and field
163 experiments around the time of herbicide application was 52 and 32%, respectively.

164 Both greenhouse and field experiments were laid out in RCBD and treatments were
165 replicated four times. The experiments were conducted twice in the greenhouse and once in
166 the field. *A. palmeri* seedlings were visually rated for overall control at 21 DAT on a scale of
167 0 to 100% for no visible effect and total death, respectively. The herbicide interaction was
168 evaluated using the equation described by Colby[26] 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[25,27]. 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.

180

181 **3. RESULTS AND DISCUSSION**

182

183 **3.1 Response to Alternative Herbicides in the Greenhouse**

184 All 12 *A. palmeri* populations used in this research had some GR individuals ranging from 14
185 - 99% (Table 1). The Pra-C field had been in continuous GR-cotton for more than 10 years
186 and had the highest frequency of GR plants. Bioassays of these 12 *A. palmeri* populations
187 with 1x glufosinate dose found a few survivors, but those plants incurred >90% injury and
188 likely would not have recovered to produce seed (data not shown). An exception to this
189 general observation was Pra-C where an average of 29% of individuals survived the full
190 dose of glufosinate (Table 1). As expected, intrapopulation variation was manifested at
191 sublethal doses.

192

193 **3.1.1 Response to 2,4-D in Greenhouse Assays**

194 All populations tested were highly sensitive to the 1x dose of 2,4-D, which killed 96-100% of
195 plants 21 DAT and the majority of survivors were injured 99% (Table 1). Occasional lesser
196 injured plants were unlikely to reproduce. Similarly, 2,4-D also showed >90% control of GR
197 *Amaranthus tuberculatus* (tall waterhemp) in Mississippi[28], but less than 90% control was
198 also reported on GR-*A. palmeri* and *Amaranthus spinosus* L. (spiny amaranth)[29,30]. In
199 their experiments, 2,4-D controlled *A. palmeri* better than dicamba or glufosinate, similar to
200 the results of our experiments.

201

202 **3.1.2 Response to Dicamba in Greenhouse Assays**

203 The full dose of dicamba caused 80 – 99% injury on surviving plants per population, 21 DAT
204 (Table 1), but some plants showed signs of recovery. Mortality ranged from 36 - 94% across
205 populations. Pra-C and STF-A were the most tolerant to the full dose, having at least 60%
206 survivors, of which 8 and 2%, respectively, had ≤50% injury (Table 1; Fig. 1). On the other
207 hand, 11% of treated plants from Con-A survived 1x dicamba, 16% of which had <50%
208 injury. Supplemental control measures are needed to prevent these survivors from producing
209 seeds. Different responses to dicamba have been reported by other researchers. Doherty *et*
210 *al.*[31] reported 99% control of 7.5- and 15-cm GR-*A. palmeri* by 0.58 kg ha⁻¹ dicamba while
211 Johnson[32] reported a wide range of dicamba activity (61-97%) on *A. palmeri*. One major
212 reason for the large variation in these results is differential tolerance between populations.
213 Populations with more inherently tolerant individuals have higher likelihood of evolving
214 resistance[13,14].

215 **3.1.3 Response to Tembotrione in Greenhouse Assays**

216 The repetition effect was not significant; therefore, data were averaged over repetition.
217 Differential response to tembotrione among *A. palmeri* populations was evident (Table 1).

218 The average mortality per population with the full dose of tembotrione ranged from 49 – 98%
219 and the survivors incurred 80 - 99% injury, 21 DAT. In a related work, tembotrione controlled
220 91 - 96% of a sensitive *Amaranthus tuberculatus* (waterhemp) population in the
221 greenhouse[33]. The percentage of individual survivors exhibiting less than 50% injury was
222 greatest in populations STF-B (45%), STF-C (26%), and Mis-B (23%) (Fig. 2). Fewer than

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

Population	Frequency of glyphosate- resistant plants (%) ^a	Mortality at 1x dose of glufosinate (%) ^b	2,4-D ^c		Dicamba ^c		Tembotrione ^c	
			Injury (%) ^d	Mortality (%)	Injury (%) ^d	Mortality (%)	Injury (%) ^d	Mortality (%)
Con-A	95	94	99	98	97	89	95	73
Cri-C	36	92	99	98	99	89	97	86
Law-B	16	93	100	100	99	94	94	64
Law-C	14	85	99	99	99	88	97	82
Lee-A	88	100	100	100	99	91	99	98
Mis-B	50	91	99	97	97	73	88	70
Mis-C	57	90	100	100	99	73	89	49
Phi-B	17	100	99	99	99	81	93	67
Pra-C	99	71	99	96	80	36	95	79
STF-A	83	100	100	100	85	36	94	70
STF-B	39	95	99	98	98	80	80	61
STF-C	90	88	99	99	98	79	84	64
LSD _{0.05} ^e	-	-	NS ^f	1	3	7	7	7

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

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

227 ^c2,4-D and dicamba in kg ae/ha; tembotrione in kg ai/ha; 1x doses for 2,4-D, dicamba, and tembotrione are 1.06, 0.56, and 0.094 kg/ha,
228 respectively.

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

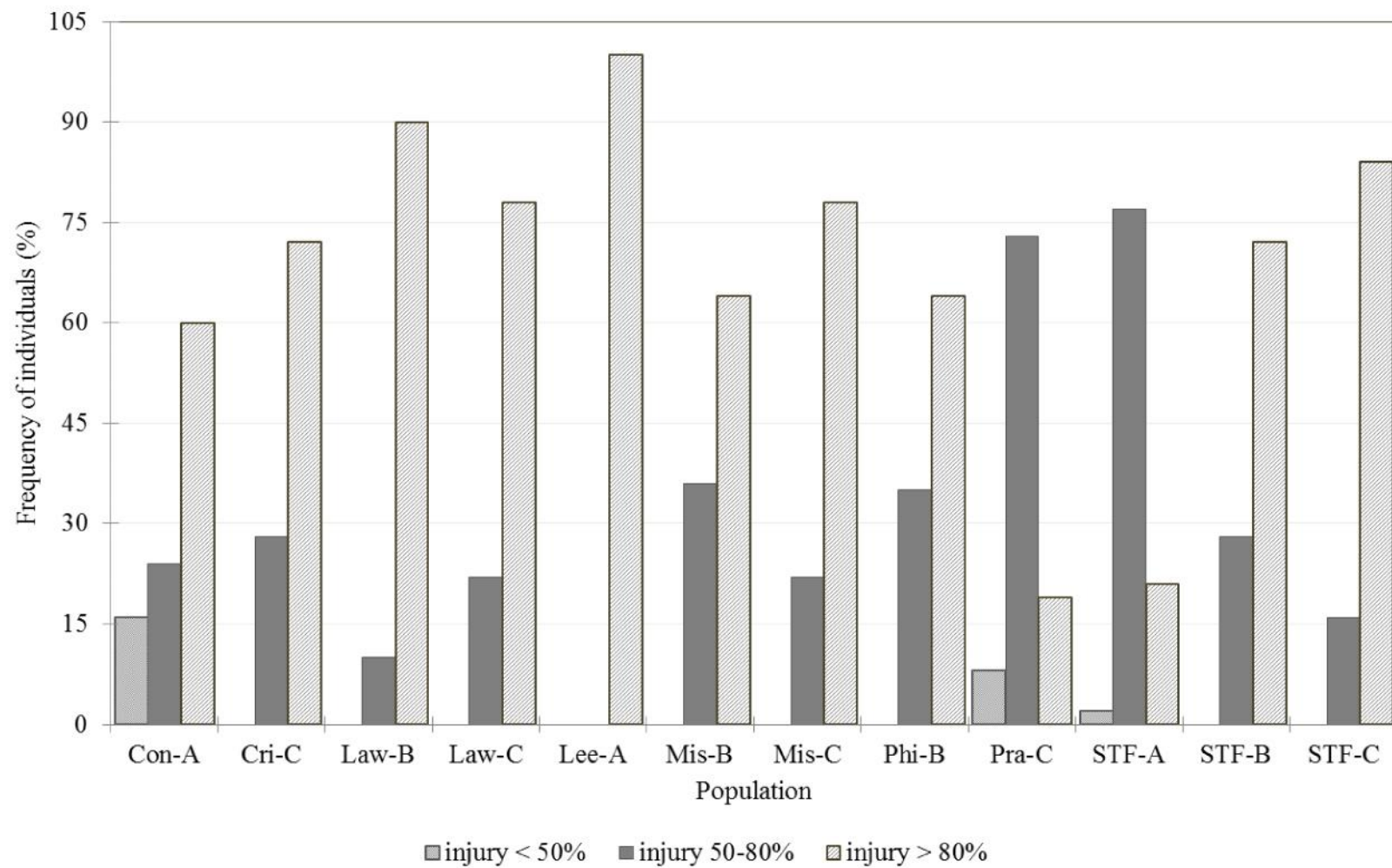
231 ^eFisher's Protected LSD to compare population means.

232 ^fNot significant.

233

234

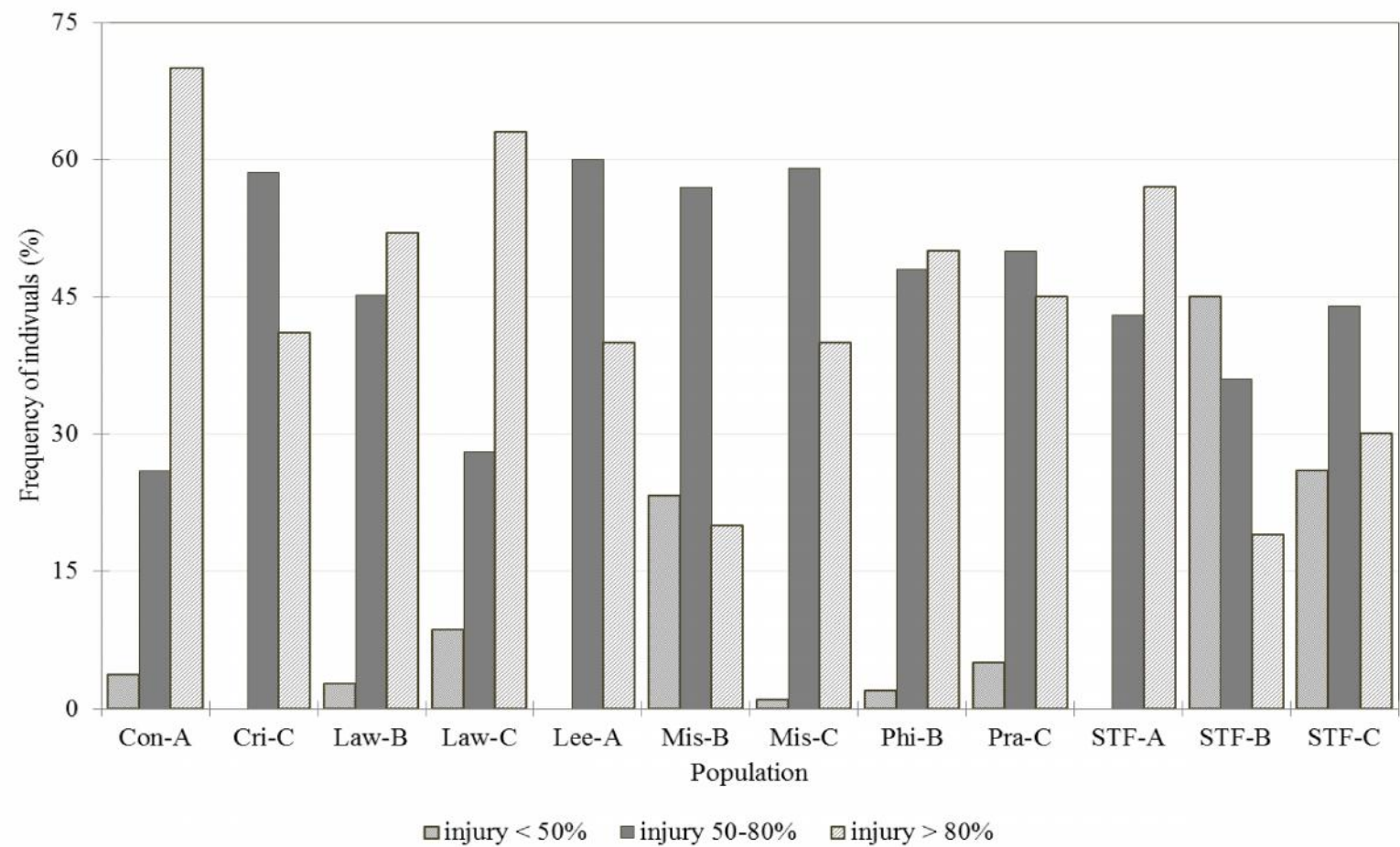
235 Figure 1
236



237

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238 Figure 2



239
240

241
242 10% of surviving plants from populations Con-A, Law-B, Law-C, Mis-C, Phi-B, and Pra-C
243 exhibited $\leq 50\%$ injury. No survivors from Cri-C, Lee-A, and STF-A had $< 50\%$ injury
244 indicating that, if such plants were in the field under reduced competition from other weeds
245 and the crop, some of these survivors could produce seed. On the other hand, all survivors
246 in Lee-A and Cri-C had $\geq 50\%$ injury and were not likely to reproduce. Populations STF-B,
247 STF-C, and Mis-B would need additional control measures to prevent seed production. The
248 most tolerant plants had new growth with no injury symptoms 14 DAT. Having 18 - 27% of
249 Mis-B, STF-C, and STF-B survivors with less than 50% injury when treated with the labeled
250 dose suggests that these populations have higher risk than others for resistance selection by
251 tembotrione.

252 Variation in population response to tembotrione was also observed between HPPD-
253 susceptible- and -resistant ecotypes. An experiment by Lally[34], in the greenhouse,
254 showed 100% control of susceptible *A. palmeri* with $0.094 \text{ kg ai ha}^{-1}$ tembotrione, but only 66
255 - 82% control of HPPD-resistant populations. Plant size and environmental differences
256 during seedling development and around the time of herbicide application are critical
257 determinants of herbicide efficacy. In the field, Lally[34] observed even lower activity of
258 tembotrione on *A. palmeri* (40 - 63% control) primarily because the plants were 15-27 cm tall
259 at application time; the label recommends spraying the herbicide when *Amaranthus* species
260 are $< 6\text{-cm}$ tall[35]. When sprayed at the correct size, 90 - 100% control of *A. palmeri* was
261 achieved with 0.094 kg ha^{-1} tembotrione[36].

262 3.2 Glufosinate Interaction with Alternative Herbicides

263 When applied separately in the field, no difference between 0.5x and 1x doses of dicamba
264 on *A. palmeri* injury was observed, but full doses of tembotrione, 2,4-D, and glufosinate
265 performed better than 0.5x doses of each (Table 2). The 1x doses of glufosinate and 2,4-D
266 had similar activity on *A. palmeri* (91 and 93%, respectively) and greater activity than the 1x
267 doses of dicamba (83%) and tembotrione (69%). Although tembotrione applied at the 1x
268 dose was as effective as glufosinate, 2,4-D, and dicamba in the greenhouse (Table 3), it had
269 the least control of *A. palmeri* in the field (Table 2).

270 Generally, the efficacy of 1x doses of glufosinate, dicamba, and tembotrione was lower in
271 the field than in the greenhouse. Unlike plants in the field, plants in the greenhouse are
272 exposed to less solar radiation and fewer physical or environmental stresses. Greenhouse-
273 grown plants are not hardened; therefore, are more susceptible to herbicides. The field
274 environment is associated with heat stress, low RH and sometimes low soil moisture
275 content, with intense solar radiation. Physiologically, these are preconditions for the
276 synthesis of thicker leaf cuticles and lignification of structural organs, which reduce herbicide
277 absorption[37,38,39,40]. Anderson *et al.*[41] reported a reduction in activity of glufosinate
278 from 100% control of *Setaria viridis* L. (green foxtail) at 95% RH to 30% growth inhibition at
279 40% RH. The mean RH for our greenhouse and field experiments was 52 and 32%,
280 respectively. At higher RH, herbicide spray droplets evaporate slower; meanwhile the leaf
281 cells are turgid and stomates are open (granting that plants are not water-stressed), overall
282 allowing maximum absorption of herbicide[39]. Thus, higher RH favors good activity of foliar
283 herbicides.

284

285

286 **Table 2. Efficacy and compatibility of glufosinate tank-mixed with tembotrione, 2,4-D**
 287 **or dicamba on *Amaranthus palmeri* under field conditions, Kibler, Arkansas, USA**
 288 **2012.**
 289

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 -----			

290 ^aTembotrione 1x dose = 0.094 kg ai/ha; 2,4-D 1x dose = 1.06 kg ae/ha; dicamba 1x dose =
 291 0.56 kg ae/ha; glufosinate 1x dose = 0.73 kg ai/ha.

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

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

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

300
 301

Table 3. 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 -----			

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

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

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

^dValues in parentheses, calculated by Colby (1967) method using the raw data, indicate the expected activity from tank mixtures having significant differences between the observed and the expected at $P = .05$. A negative sign (-) denotes an antagonistic and a positive (+) denotes synergistic response.

3.2.1 Glufosinate interaction with 2,4-D

In the field and greenhouse, we observed favorable interaction between glufosinate and 2,4-D for the control of *A. palmeri* population PRA-C (Tables 2 and 3). In the field, glufosinate alone at the 1x dose controlled PRA-C 91%; the addition of 0.25x 2,4-D improved weed control to 94% while the addition of 0.5x 2,4-D attained 100% control (Table 2). Interestingly, mixing half doses of 2,4-D and glufosinate resulted in 100% control, demonstrating additive effects according to Colby's test[26]. Synergistic interaction was observed when 0.25x dose of glufosinate (0.18 kg ai/ha) was mixed with 0.25x or 0.5x dose of 2,4-D. The synergistic mixture of 0.25x glufosinate with 0.5x 2,4-D resulted in 96% control of *A. palmeri*, which is excellent for commercial crop production although not favorable for long-term resistance management. The goal should be 100% control[11]. 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 3). Combinations of any dose of 2,4-D with at least 0.5x dose of glufosinate resulted in 100% control of *A. palmeri*.

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)[42]. 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 mixtures should also be used in conjunction with all other weed management practices.

3.2.2 Glufosinate Interaction with Dicamba

Under field conditions the mortality of PRA-C population with the 1x dose of dicamba alone was 83% (Table 2) and 100% in the greenhouse (Table 3). Antagonism was observed both in the field and greenhouse when <1x glufosinate dose was mixed with 0.5x dose of dicamba (Tables 2 and 3) or, when 0.25x glufosinate was mixed with 1x dose of dicamba in the field (Table 2). 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 manage GR-A. *palmeri*. The efficacy of herbicide combinations need to be tested because interactions of different chemistries differ and these are further modified by dose and species [24,25,27,42,43]. 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*[42].

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 both in the field (Table 2) and the greenhouse (Table 3). 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.*[43] 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[44]. 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.

4. CONCLUSION

The glyphosate-resistant *A. palmeri* populations tested respond differentially to tembotrione, 2,4-D, and dicamba. The PRA-C population had higher tolerance to both 2,4-D and dicamba than the other populations. Generally, only a small percentage of GR-*A. palmeri* survived the full dose of 2,4-D or dicamba. Nevertheless, the survivors should be prevented from producing seed, using supplemental weed control practices. Otherwise, we predict that selection for resistance to dicamba will occur more quickly than that with 2,4-D because of higher frequency of survivors with dicamba. Similarly, higher dose of tembotrione, sequential application, or compatible tank mixtures are needed to maximize *A. palmeri* control with this herbicide. The majority of glufosinate + tembotrione and some glufosinate + dicamba tank mixtures were antagonistic in the field. Among the alternative herbicides, tembotrione was the least beneficial tank-mix 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 **before being integrated in a management program**.

ACKNOWLEDGEMENTS

The authors acknowledge the help of Dennis Motes and crew at the Vegetable Research Station, Kibler, Arkansas; Vaughn Skinner and crew at the Arkansas Main Research Center, Fayetteville; Leopoldo Estorninos, Vijay Singh, Caroline Bevilacqua, Ana Carolina Roso, and Fernando Martini. This research was supported by the Arkansas Soybean Board and Bayer Crop Science.

405 **COMPETING INTERESTS**

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407 The authors have no competing interests.

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410 **AUTHORS' CONTRIBUTIONS**

411 Author GMB, conducted the experiment, collected and analyzed data, prepared the first draft
412 of the manuscript and worked with NRB in revising the manuscript. NRB collected the plant
413 samples from grower fields together with EAA, designed the experiment, revised and
414 finalized the manuscript. EEG guided the data analysis and interpretation. EAA and RAS
415 helped in the experiment implementation and data collection. RCS contributed to the
416 finalization of treatments and guided the practical interpretation of results. All authors
417 approved the manuscript.

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540

541 **FIGURE LEGENDS**

542 Figure 1. Injury frequency distribution of *A. palmeri* plants surviving 0.56 kg ae/ha dicamba in
543 greenhouse experiments. Visual rating was conducted 21 days after treatment. Dicamba
544 was applied to 7-cm-tall seedlings in 187 L/ha spray volume. Visual rating was conducted 21

545 days after treatment. The data set was comprised of individual ratings of 200 plants per
546 population (4 replications, 25 plants per replication, 2 repetitions).

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

553