

Research Paper

**Synergistic interaction of sublethal doses of 2,4-D
and glufosinate on glyphosate-resistant *Amaranthus
palmeri***

ABSTRACT

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

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

Place and Duration of Study: Department of Crop, Soil, and Environmental Sciences, University of Arkansas-Fayetteville, USA. Tolerance experiments were conducted in October–November 2010 and in July–August 2011. The herbicide interaction experiments were conducted in the greenhouse in June–July 2011 and August–September 2011. This was also done in the field in April–August 2012.

Methodology: Herbicide treatments in the tolerance experiment were 0.024-0.094 kg/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 was evaluated at 0.18-0.73 kg/ha and the tank-mix options were 0.02-0.094 kg/ha tembotrione, 0.28-1.12 kg ae/ha 2,4-D; and 0.28 and 0.56 kg

ae/ha dicamba. The population used was PRA-C, which had 20% survival to a field dose of glufosinate at 0.73 kg/ha.

Results: Tembotrione (0.094 kg/ha) and dicamba (0.56 kg/ha) controlled *A. palmeri* 80-100%; 2,4-D at 1.06 kg/ha controlled all populations 99-100%. These were field use doses. In the greenhouse and field, mixing half doses of glufosinate and 2,4-D resulted in 100% control. The full dose of glufosinate controlled *A. palmeri* 91% in the field; addition of a half or full dose of 2,4-D or a full dose of dicamba resulted in 100% control.

Conclusion: Under field conditions, 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 in the greenhouse and field.

Keywords: *Differential tolerance; herbicide resistance; HPPD inhibitor; resistance management; selection; tank mixing.*

1. INTRODUCTION

Of all the *Amaranthus* species in the southern US, *Amaranthus palmeri* L. (Palmer amaranth) is the most prolific, competitive, and common weed in crop fields. 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^[1,2,3,4], *A. palmeri* also interferes with production and processing activities such as harvesting and ginning cotton (*Gossypium hirsutum* L.)^[5]. Historically, *A. palmeri* has been controlled with various herbicides with 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^[6]. 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^[7]. Thus, resistance to glyphosate has evolved in an increasing number of weed species, including *A. palmeri*. The rapid increase in glyphosate-resistant weed populations^[8] 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^[9]. 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^[8]. 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^[8,10,11]. 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, hydroxyphenylpyruvatedioxygenase (HPPD) inhibitors, and possibly to the protoporphyrinogen oxidase (PPO) inhibiting herbicides are expected to be commercialized in the near future^[12,13]. Scientists and crop growers 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^[14,15]. Variability in weed population response to herbicides has also been reported in other species^[16,17]. 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 spectrum controlled and ensures season-long weed control if residual herbicides are included in the mixture^[18,19,8,20]. 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^[22,23,24,25]. 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 *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.

100

101 2. MATERIALS AND METHODS

102 2.1 *A. palmeri* Response to Alternative Herbicides

103 Greenhouse experiments were conducted between October 2010 and July 2011 at the
 104 Altheimer greenhouses, Fayetteville, Arkansas, to evaluate the response of 12 *A. palmeri*
 105 populations to tembotrione, 2,4-D, and dicamba. Seeds were collected from 10 to 20 plants
 106 surviving at least two 0.84 kg ae/ha glyphosate applications in the 2009 growing season from
 107 growers' fields in Mississippi, Lee, Lawrence, Phillips, Conway, St Francis, Prairie, and
 108 Poinsett counties, Arkansas. For general testing of resistance to glyphosate, from each
 109 individually sampled plant, 100 offsprings were grown in individual cells in trays (Redwayfeed
 110 Garden and Pet supply, 290 Briceland Rd, Reedway, CA 95560) filled with Sunshine premix
 111 soil (Sunshine premix #1[®], Sun Gro Horticulture, 15831 NE 8th Street, Suite 100, Bellevue,
 112 WA 98008). The trays were placed in the greenhouse with 34/26(±4) °C day/night
 113 temperatures. At the two-leaf stage, seedlings were thinned to one plant per cell and sprayed
 114 with 840 g ae/ha glyphosate at three-to-four-leaf stage. The herbicide was applied using a
 115 motorized boom equipped with two nozzles (TeeJet[®] 800067 flat-fan spray nozzles, Spraying
 116 Systems Co., P.O. Box 7900, Wheaton, IL 60189) spaced 51 cm apart and calibrated to
 117 deliver 187 L/ha at 262 kPa. At 21 days after treatment (DAT) the number and visible injury
 118 of survivors were evaluated, using a scale of 0 (no injury) to 100% (complete death). The
 119 assay was repeated.

120

121 To test differential response of populations to alternative herbicides, 500 mg of seeds from
 122 each plant were mixed to make a composite subsample. Seeds from the composite were
 123 planted in cellular trays using the same procedure as in the glyphosate assay. When
 124 seedlings were about 7-cm tall, tembotrione (Laudis[®], Bayer CropScience, 26 Davis Drive,

Research Triangle Park, NC 27709, USA), 2,4-D (Weedar 64[®], Nufarm Inc., 150 Harvester Drive, Burr Ridge, IL, 60527, USA), or dicamba (Clarity[®], 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 0.25x, 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. Ammonium sulfate (AMS) was added at 10 g/L to tembotrione. Methylated seed oil was also added to all tembotrione, 2,4-D and dicamba treatments at 1% v/v. The treated seedlings were returned to the greenhouse and maintained for 21 days when the evaluation was conducted.

A. palmeri response to the alternative herbicides was evaluated in separate experiments. The experimental design was randomized complete block (RCBD) 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 control) to 100% (complete control) 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 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 in the 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 20% survival frequency to the labeled dose of glufosinate in previous assays. *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); dicamba at 0.5x or 1x (0.56 kg ae/ha); or 2,4-D at 0.25x, 0.5x or 1x (1.06 kg ae/ha). Ammonium sulfate was added at 10 and 20 g/L to tembotrione-alone and glufosinate-alone treatments, respectively. Methylated seed oil was also added to all tembotrione, 2,4-D and dicamba 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 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 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) spaced 51 cm apart and calibrated to deliver 187 L/ha at 248 kPa. The mean RH for the greenhouse and field experiments was 52 and 32%, respectively.

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176 Both greenhouse and field experiments were laid out in RCBD and treatments were
177 replicated four times. The experiments were conducted twice in the greenhouse and once in
178 the field. *A. palmeri* seedlings were visually rated for injury 21 DAT on a scale of 0 to 100%
179 for no control and total death, respectively. The herbicide interaction was evaluated using the
180 equation described by Colby (1967) shown below;

181

$$182 \quad E_1 = (X_1 + Y_1) - (X_1 Y_1 / 100) \quad (1).$$

183

184 Where E_1 is the expected control from the combination of herbicides A and B; X_1 and Y_1 are
185 the observed control from herbicide A and B alone, respectively. The expected control from
186 the tank mixture was calculated using observed control values from individual herbicides
187 used in the mixture within each replicate. The expected control (E_1) was then compared with
188 the observed control obtained from the tank mix using Fisher's Protected LSD (Hicks *et al.*,
189 1998; Koger *et al.*, 2007). The interaction was considered to be synergistic if the observed
190 plant response to the herbicide mixture was significantly higher than the expected, or
191 antagonistic if the observed control was significantly lower than the expected, or additive if
192 the observed and the expected responses were similar. The injury and mortality data were
193 subjected to ANOVA using PROC GLM in SAS and means were separated by Fisher's
194 protected LSD at the 5% level of significance.

195

196 **3. RESULTS AND DISCUSSION**

197 **3.1. Response to Herbicides in the Greenhouse**

198 Tolerance of *A. palmeri* populations differed within and across herbicide doses for
199 tembotrione, 2,4-D and dicamba. As expected, responses to herbicides were more variable

at the lower than at higher doses. At the 1x dose, response variability was least with 2,4-D and highest with tembotrione. The response variability increased as the doses decreased. Intrapopulation variation is manifested more at sublethal doses, demonstrating that using reduced rates of herbicides is not a good management practice unless other supplementary control measures (allelopathic cover crops, green manure, cultivation, handweeding) are used.

3.1.1 Response to Tembotrione in Greenhouse Assays

No interactions involving population and tembotrione dose were observed. The run effect was not significant; therefore, data were averaged over runs. Differential response to tembotrione among *A. palmeri* populations was evident at all doses but was most pronounced at lower doses of 0.25x and 0.5x. The full dose of tembotrione injured *A. palmeri* 80-100%, 21 DAT (Table 1). At the 1x dose, Mis-B, STF-B and STF-C showed less injury than the other populations. The survival rate of plants at the 1x dose of tembotrione ranged from <1% in Lee-A to 51% in Mis-C populations (Table 2). At the 0.5x dose of tembotrione, only one population had >50% mortality. 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, Pra-C, and STF-A had less than 10% of survivors with ≤50% injury. On the other hand, all survivors in Lee-A and Cri-C had ≥50% injury and were not likely to produce seeds. Populations STF-B, STF-C, and Mis-B would need additional control strategy to prevent seed production. Most treated plants of these tolerant populations 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.

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226 Variation in population response to tembotrione was also observed between HPPD-
227 susceptible- and resistant ecotypes. An experiment by Lally (2011)^[29], in the greenhouse,
228 showed 100% control of susceptible *A. palmeri* with 0.094 kg ai ha⁻¹ tembotrione, but only
229 66-82% control of HPPD-resistant populations. Plant size and environmental differences
230 during seedling development and around the time of herbicide application are critical
231 determinants of herbicide efficacy. In the field, Lally (2011)^[29] observed even lower activity of
232 tembotrione on *A. palmeri* (40-63% control) primarily because the plants were 15-27 cm tall
233 at application time; the label recommends spraying the herbicide when *Amaranthus* species
234 are <6-cm tall^[30]. When sprayed at the correct size, 90–100% control of *A. palmeri* was
235 achieved with 0.094 kg ha⁻¹ tembotrione^[31].

236

237 **3.1.2 Response to 2,4-D in Greenhouse Assays**

238 Due to minimal or no variance among replicates, the data were not subjected to analysis of
239 variance. 2,4-D applied at 1x controlled all populations 99-100% (Table 1). Mortality ranged
240 from 98-100% and was influenced by herbicide dose and *A. palmeri* population (Table 2).
241 The few surviving plants had at least 80% injury at the time of evaluation, and were unlikely
242 to reproduce. 2,4-D also showed >90% control of GR *Amaranthus tuberculatus* (tall
243 waterhemp) in Mississippi^[32], but less than 90% control was also reported on GR-*A. palmeri*
244 and *Amaranthus spinosus* L. (spiny amaranth)^[33,34]. In their experiments, 2,4-D controlled of
245 *A. palmeri* better than did dicamba or glufosinate, similar to what we observed in our
246 experiments.

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248 **3.1.3 Response to Dicamba in Greenhouse Assays**

Dicamba dose by population interaction effect on *A. palmeri* control was significant ($P < 0.001$), but not on mortality ($P = 0.14$). The full rate of dicamba controlled *A. palmeri* populations 84-100% at 21 DAT (Table 1), but some plants showed signs of recovery. The 2x dose of dicamba did not kill all plants in the less sensitive populations. The use of 2x rate, of course, is not recommended. Mortality ranged from 36-97% across populations, with Pra-C and STF-A being the most tolerant, having at least 60% survivors, of which 8 and 2%, respectively, had $\leq 50\%$ injury (Table 2; Fig. 2). On the other hand, 11% of treated plants from Con-A survived 1x dicamba, 16% of which had $< 50\%$ injury. Supplemental control measures are needed to prevent these survivors from producing seeds. Different responses to dicamba have been reported by other researchers. Doherty *et al.* (2010)^[35] reported 99% control of 7.5- and 15-cm GR-*A. palmeri* by 0.58 kg ha⁻¹ dicamba while Johnson (2011)^[36] reported a wide range of dicamba activity (61-97%) on *A. palmeri*. One major reason for the large variation in these results is differential tolerance between populations. Populations with more inherently tolerant individuals have higher likelihood of evolving resistance^[10,11].

3.2 Glufosinate Interaction with Alternative Herbicides

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

Generally, the efficacy of 1x doses of glufosinate, dicamba, and tembotrione was lower in the field than in the greenhouse. Unlike plants in the field, plants in the greenhouse are exposed to less solar radiation and are not exposed to physical or environmental stresses. Greenhouse-grown plants are not hardened; therefore, are more susceptible to herbicides. The field environment is associated with heat stress, low relative humidity (RH) and sometimes low soil moisture content, with intense solar radiation. Physiologically, these are preconditions for the synthesis of thicker leaf cuticles and lignification of structural organs, which reduce herbicide absorption^[37,38,39,40]. Anderson *et al.* (1993)^[41] reported a reduction from 100% control of *Setaria viridis* L. (green foxtail) at 95% RH to 30% growth inhibition at 40% RH. The mean RH for our greenhouse and field experiments was 52 and 32%, respectively. At higher RH, herbicide spray droplets evaporate slower; meanwhile the leaf cells are turgid and stomates are open (granting that plants are not water-stressed), overall allowing maximum absorption of herbicide. Thus, higher RH favors good activity of foliar herbicides.

3.2.1 Glufosinate interaction with 2,4-D

The data showed favorable interaction between glufosinate and 2,4-D for the control of *A. palmeri* population PRA-C. As the full dose of glufosinate controlled *A. palmeri* only 91%, the addition of 0.25x 2,4-D improved weed control to 94%; the addition of 0.5x 2,4-D attained 100% control. 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 the lowest 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 already excellent for commercial crop production although not favorable for long-term resistance management. The goal should be 100% control^[8] and

in this research, this was achieved with the mixture of 0.5x or 1x doses of glufosinate and 2,4-D. 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.

3.2.3 Glufosinate Interaction with Tembotrione

Tank mixing 1x doses of glufosinate with 0.25 or 0.5x dose of tembotrione antagonized the activity of each herbicide thereby reducing *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. Synergistic or antagonistic interaction of glufosinate with other herbicides in the tank mixture has been reported previously. Burke *et al.* (2005)^[42] observed that the activity of clethodim applied at 105 or 140 g/ha on *Eleusine indica* L. (goosegrass) was antagonized by at least 52% points when mixed with 290 or 410 g/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/ha glufosinate was mixed with clethodim, fluazifop-P, quizalofop-P, or sethoxydim^[43]. 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.

332

333 **4 CONCLUSIONS**

Glyphosate-resistant *A. palmeri* populations respond differentially to tembotrione, 2,4-D, and dicamba. Pra-C population has higher tolerance to both 2,4-D and dicamba than the rest. Generally, only a small percentage of GR-*A. palmeri* evaluated can survive 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 quickly. Similarly, higher rates of tembotrione, sequential application, or compatible tank mixtures are needed to maximize *A. palmeri* control. Of the three herbicides, tembotrione has the highest risk of selecting for resistant populations. The majority of glufosinate + tembotrione and some glufosinate + dicamba tank mixtures were antagonistic. Among the alternative herbicides, tembotrione is the least favorable tank-mix partner with glufosinate 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.

348

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356 **COMPETING INTERESTS**

357 The authors have no competing interests.

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

Population	Frequency of glyphosate-resistant plants (%) ^a	<i>Amaranthus palmeri</i> control (%) ^{b,c}							
		tembotrione			2,4-D			dicamba	
		0.024	0.047	0.094	1.06	2.12	0.56	1.12	
Con-A	95	12.1	73.8	95.3	99.5	100	97.4	98.9	
Cri-C	36	12.3	72.2	97.0	99.5	100	99.3	98.9	
Law-B	16	17.3	69.4	93.5	100.0	100	99.9	99.1	
Law-C	14	11.5	83.5	96.9	99.7	100	99.4	98.6	
Lee-A	88	23.6	81.9	99.6	100.0	100	99.8	98.8	
Mis-B	50	29.6	77.3	87.8	99.3	100	97.1	98.7	
Mis-C	57	15.1	69.1	89.0	100.0	100	98.6	98.1	
Phi-B	17	14.5	71.2	92.7	99.5	100	98.9	98.9	
Pra-C	99	12.3	62.8	95.1	99.2	100	79.6	98.2	
STF-A	83	16.9	60.4	94.1	100.0	100	85.4	98.8	
STF-B	39	13.7	75.3	80.0	99.5	100	98.4	98.4	
STF-C	90	13.1	70.5	84.5	99.7	100	98.3	99.1	
LSD _{0.05} within herbicide ^d		6.7			_f			3.4	

Within herbicide and dose ^e	6.3		-		3.0
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479 ^aA total of 200 seedlings per population were sprayed with glyphosate, 0.84 kg ae/ha, at the
480 3-4 leaf stage.

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

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

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

486 ^eLSD to compare population means within the same herbicide dose.

487 ^fData for 2,4-D were not subjected to ANOVA due to minimal or no variance among
488 replicates and runs.
489

490 **Table 1. Mortality of 12 *Amaranthus palmeri* populations 21 days after tembotrione,**
491 **2,4-D and dicamba treatments in greenhouse experiments.**

Population	<i>A. palmeri</i> mortality (%) ^{a,b}						
	tembotrione			2,4-D		dicamba	
	0.024	0.047	0.094	1.06	2.12	0.56	1.12
Con-A	0	38.5	73.0	97.9	100.0	89.3	97.1
Cri-C	0	32.5	85.5	97.9	99.5	89.0	95.6
Law-B	0	37.5	63.5	99.7	100.0	93.9	95.6
Law-C	0	47.0	82.5	99.0	100.0	88.5	94.1
Lee-A	0	46.5	97.5	100.0	100.0	91.0	94.6
Mis-B	0	43.0	70.0	97.0	99.7	72.8	92.5
Mis-C	0	34.0	49.0	99.7	100.0	73.4	93.1
Phi-B	0	27.5	67.0	99.0	99.2	80.5	94.0
Pra-C	0	41.0	79.0	96.1	99.7	36.5	73.8
STF-A	0	20.0	70.0	99.7	100.0	36.0	86.0
STF-B	0	44.0	61.0	97.6	100.0	79.5	92.8
STF-C	0	54.0	63.5	99.2	100.0	79.0	93.5
LSD _{0.05}							
Within herbicide ^c		6.8		0.5		6.8	
Within herbicide and dose ^d		13.6		₋ ^f		13.6	

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

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

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

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

499 ^fData for 2,4-D were not subjected to ANOVA due to minimal or no variance among
500 replicates and runs.

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

Tank-mix option	Dose (kg/ha) ^a	<i>A. palmeri</i> control (%) ^b			
		glufosinate dose (kg ai/ha)			
		0 ^c	0.18	0.37	0.73
None	0.00	0.0	38.3	83.8	90.9
Tembotrione	0.024	17.6	52.7	55.7 (86.7)-	60.1 (93.1)-
Tembotrione	0.047	59.4	55.7 (86.7)- ^d	73.2 (94.3)-	77.7 (96.7)-
Tembotrione	0.094	69.4	60.1 (93.2)-	93.3 (97.0)-	91.5 (98.5)-
2,4-D	0.27	43.3	79.9 (64.7)+	87.6	94.4
2,4-D	0.53	75.6	95.8 (84.4)+	100.0	100.0
2,4-D	1.06	93.3	91.7	100.0	100.0
Dicamba	0.28	83.3	66.7 (88.9)-	81.3 (94.3)-	94.4
Dicamba	0.56	83.3	76.4 (87.5)-	93.3	100.0
^c LSD _{0.05}		-----2.8-----			

^aTembotrione dose in kg ai/ha; 2,4-D and dicamba doses in kg ae/ha.

^bInjury based on visual rating 21 days after treatment on a scale of 0 to 100%; 0 = no injury, 100 = total death. Each value is the average of 4 replicates, 25 plants/replicate.

^cFisher's Protected LSD to compare treatment means within the same columns 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 $P = 0.05$. A negative sign (-) denotes an antagonistic interaction; a positive (+) sign denotes synergistic interaction.

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

Tank-mix option	Dose ^a (kg/ha)	<i>A. palmeri</i> control (%) ^b			
		glufosinate dose (kg ai/ha)			
		0	0.18	0.37	0.73
None		0.0	69.8	93.7	99.4
Tembotrione	0.024	64.1	65.5 (90.9)- ^d	78.5 (99.1)-	92.1 (99.9)-
Tembotrione	0.047	93.4	89.6 (98.4)-	96.8 (99.8)-	93.8 (100.0)-
Tembotrione	0.094	97.9	96.8 (99.6)-	99.7 (100.0)-	99.6
2,4-D	0.27	88.3	98.6	99.9	100.0
2,4-D	0.53	96.8	98.9	100.0	100.0
2,4-D	1.06	99.8	100.0	100.0	100.0
Dicamba	0.28	92.8	95.0 (98.0)-	96.0 (99.7)-	100.0
Dicamba	0.56	99.6	99.8	99.9	100.0
^c LSD _{0.05}		-----5.5-----			

518 ^aTembotrione rate in kg ai/ha; 2,4-D and dicamba rates in kg ae/ha.

519 ^bInjury based on visual rating 21 days after treatment on a scale of 0 to 100%; 0 = no injury,
 520 100 = total death; n = 200.

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

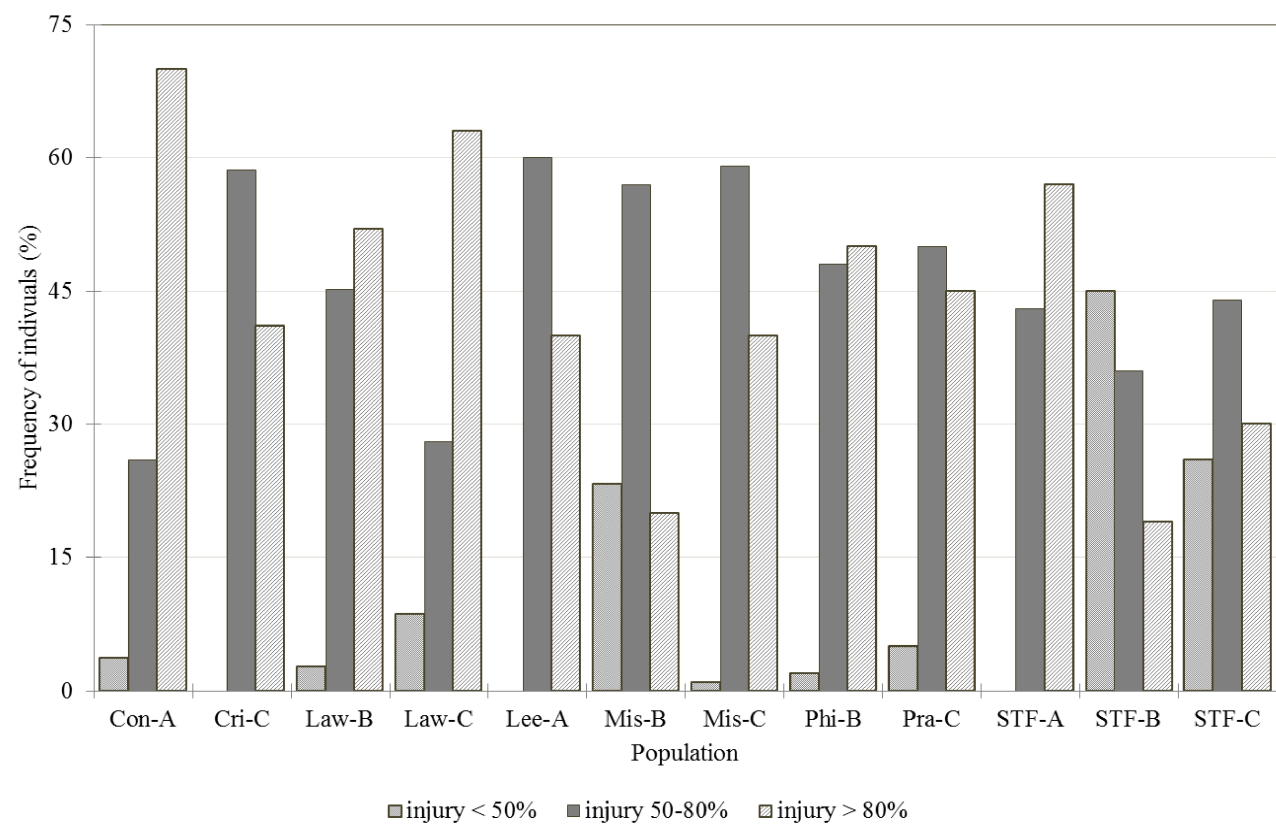
522 ^dValues in parentheses, calculated by Colby (1967) method using the raw data, indicate the
 523 expected activity from tank mixtures having significant differences between the observed and
 524 the expected at $P = 0.05$. A negative sign (-) denotes an antagonistic and a positive (+)
 525 denotes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

526

Figure Legends

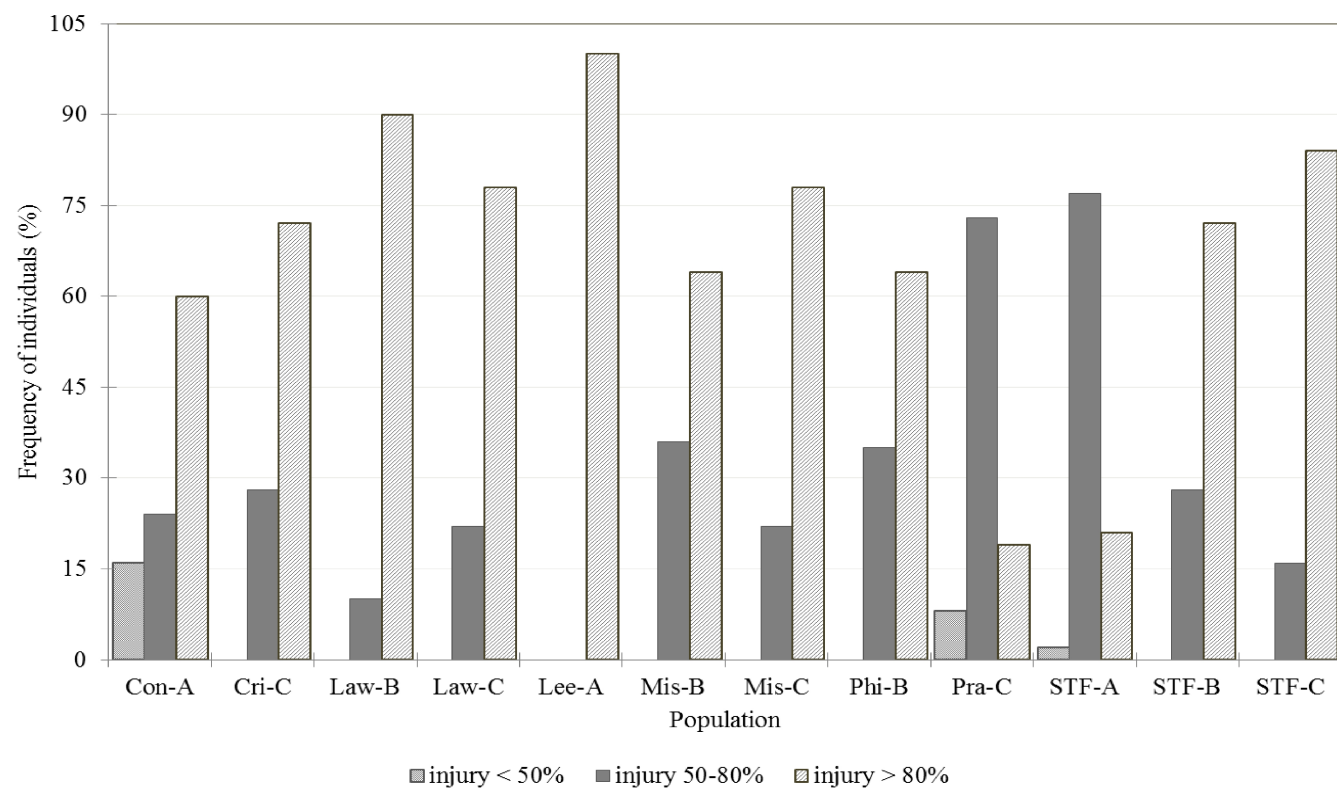
Figure 1. Injury frequency distribution of *A. palmeri* plants surviving 0.094 kg ai/ha tembotrione in greenhouse experiments. Tembotrione was applied, to 7-cm-tall seedlings, with 10 g/L ammonium sulfate and 1% by volume methylated seed oil, in 187 L/ha spray volume. Visual rating was conducted 21 days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

Figure 2. Injury frequency distribution of *A. palmeri* plants surviving 0.56 kg ae/ha dicamba in greenhouse experiments. Visual rating was conducted 21 days after treatment. Dicamba was applied, to 7-cm-tall seedlings, with 1% by volume methylated seed oil in 187 L/ha spray volume. Visual rating was conducted 21 days after treatment. The data set was comprised of individual ratings of 200 plants per population (4 replications, 25 plants per replication, 2 runs).



541

542 Figure 1



543

544 Figure 2