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# Multiple Resistance in Palmer Amaranth to Glyphosate and Pyriithiobac Confirmed in Georgia

Lynn M. Sosnoskie, Jeremy M. Kichler, Rebekah D. Wallace, and A. Stanley Culpepper\*

In 2006, Palmer amaranth with confirmed resistance to glyphosate (GLY-R) was not controlled effectively in cotton with pyriithiobac, an acetolactate synthase (ALS)-inhibiting herbicide. Glyphosate at 870 g ae ha<sup>-1</sup> or pyriithiobac at 70 g ai ha<sup>-1</sup> applied postemergence provided 5 to 28% control of a putative GLY/ALS-R Palmer amaranth biotype in the field. Glyphosate at 6,930 g ha<sup>-1</sup> and pyriithiobac at 420 g ha<sup>-1</sup> applied alone provided no more than 89 and 65% control 1 to 8 wk after treatment (WAT), respectively. When applied as a tank mixture, glyphosate plus pyriithiobac at 870 + 70 g ha<sup>-1</sup> provided between 16 and 41% control; glyphosate plus pyriithiobac at 6,930 + 420 g ha<sup>-1</sup> controlled the Palmer amaranth in the field 89 to 95%. Dose-response analyses developed from greenhouse data indicated that the estimated glyphosate rates required to produce 50% injury and reduce plant fresh weights by 50% relative to the nontreated control in a suspected GLY/ALS-R Palmer amaranth biotype were 12 and 14 times greater, respectively, than the estimated values for the susceptible (S) biotype. The predicted pyriithiobac rates required to produce the same responses in the putative resistant population were 151 (50% injury) and 563 times (50% fresh weight reduction) greater than the estimated rates for the S biotype. Field and greenhouse analyses confirm that the Palmer amaranth biotype evaluated in both studies is resistant to glyphosate and an ALS-inhibiting herbicide.

**Nomenclature:** Glyphosate; pyriithiobac; Palmer amaranth, *Amaranthus palmeri* S. Wats; cotton, *Gossypium hirsutum* L.

**Key words:** Herbicide resistance, weed resistance.

Palmer amaranth is a common and competitive weed of cotton in the southern United States. (Webster 2009). Morgan et al. (2001) reported that a Palmer amaranth density of 10 plants per 9.1-m row of cotton reduced cotton biomass approximately 50% at 8 wk after cotton emergence (WAE), cotton canopy volume 45% at 10 WAE, and cotton lint yield by 54%. Rowland et al. (1999) showed that for each 1-kg increase in Palmer amaranth biomass per plot, cotton lint yield was reduced between 5 and 9%. In addition to reducing yields, Morgan et al. (2001) determined that Palmer amaranth densities exceeding six plants per 9.1-m row significantly impeded mechanical harvesting of cotton. Smith et al. (2000) reported that Palmer amaranth infestations increased cotton harvest time because of slower ground speeds and an increased number of work stoppages due to lodged stems. Yield losses in response to Palmer amaranth interference have also been reported for other crops including soybean [*Glycine max* (L.) Merr.], corn [*Zea mays* L.], grain sorghum [*Sorghum bicolor* (L.) Moench ssp. *Bicolor*], and peanut [*Arachis hypogaea* L.] (Bensch et al. 2003; Burke et al. 2007; Klingaman and Oliver 1994; Massinga et al. 2001; Moore et al. 2004).

In 2004, a GLY-R biotype of Palmer amaranth was discovered in a field in Macon County, Georgia (Culpepper et al. 2006). Glyphosate at 12 times the recommended label rate of 840 g ae ha<sup>-1</sup> failed to provide commercially acceptable control of this biotype in the field (Culpepper et al. 2006). The mechanism of resistance in the Macon County biotype has since been attributed to an amplification of the 5-enolpyruvylshikimate-3-phosphate synthase gene (Gaines et al. 2010). As of 2010, 52 counties in Georgia are currently infested, to varying degrees, with GLY-R Palmer amaranth. Currently, GLY-R Palmer amaranth populations have been confirmed in 10 states (Alabama, Arkansas, Georgia, Louisiana, Missouri,

Mississippi, North Carolina, New Mexico, South Carolina, and Tennessee) (Heap 2010).

In 2002, the first occurrence of Palmer amaranth resistance to the ALS-inhibiting herbicides in Georgia was reported by Vencill et al. (2002). In 2005, an increased number of Palmer amaranth control failures in peanut associated with the ALS-inhibiting herbicide imazapic prompted a state-wide survey to determine the geographical distribution of ALS-R Palmer amaranth in Georgia (Wise et al. 2009). All of the 61 accessions evaluated were significantly more resistant to imazapic (3 to 55% control) at rates ranging from 70 to 700 g ai ha<sup>-1</sup> than the S check (100% control) (Wise et al. 2009). Experiments to evaluate cross resistance to other ALS-inhibiting herbicides indicated that 30 accessions were resistant to imazapic, chlorimuron, pyriithiobac, and diclosulam at recommended field use rates (Wise et al. 2009). Eight states, including Georgia, have Palmer amaranth populations with documented resistance to the ALS inhibitors (Heap 2010). In 2006, a population of GLY-R Palmer amaranth in Macon County, GA, was not effectively controlled by pyriithiobac at the labeled rate. The objective of this study was to determine the level of resistance to glyphosate and pyriithiobac in this putative GLY/ALS-R Palmer amaranth population.

## Materials and Methods

**Field Experiment.** The experiment was conducted in a field near Oglethorpe, GA, in 2007 and 2008. Soil at the site was a Dothan loamy sand (fine-loamy, siliceous, thermic, Plinthic Paleudults) with 1.9 to 2.1% organic matter and pH 6.2 to 6.4. From 1999 to 2004, the field had been treated with herbicides consisting only of pendimethalin, glyphosate, and paraquat. In 2004, the grower reported being unable to control Palmer amaranth with glyphosate (Culpepper et al. 2006). Pyriithiobac was applied POST by the grower to the same Palmer amaranth population in 2005 and applied both PRE and POST in 2006. In 2006, the grower reported that both glyphosate and pyriithiobac were ineffective in controlling Palmer amaranth.

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Table 1. Visual estimates of control of glyphosate/acetolactate synthase inhibiting herbicide-resistant (GLY/ALS-R) Palmer amaranth in the field by glyphosate and pyriithiobac, applied singly and tank-mixed, at 1, 3, 5, and 8 wk after treatment (WAT).

Herbicide rate g ha <sup>-1</sup>	Visual control <sup>a</sup>			
	1 WAT	3 WAT	5 WAT	8 WAT
	—%—			
Glyphosate				
870	5	5	5	5
1,730	34*	24*	23*	19*
3,470	68*	62*	47*	49*
6,930	89*	89*	80*	76*
Pyriithiobac				
70	28	31	25	12
140	39*	39	32	17
280	39*	49*	51*	28*
420	47*	58*	65*	48*
Glyphosate + pyriithiobac				
870 + 70	41	43	31	16
1,730 + 140	57*	70*	55*	48*
3,470 + 280	76*	77*	76*	71*
6,930 + 420	92*	95*	89*	90*

<sup>a</sup> Control values followed by an asterisk within each herbicide are significantly different from the level of control achieved using the field rate (glyphosate = 870 g ha<sup>-1</sup>; pyriithiobac = 70 g ha<sup>-1</sup>) as determined using contrast statements.

Cotton (DP 145 B2RF<sup>1</sup> in 2007 and DP 555 BRR<sup>1</sup> in 2008) was planted in conventionally prepared seedbeds with plots being two rows spaced 91 cm apart for 12 m. On April 27, 2007, and April 17, 2008, the entire trial area was treated with pendimethalin<sup>2</sup> at 1,117 g ha<sup>-1</sup> immediately following planting. Twelve POST applied herbicide treatments were evaluated in the study and included: glyphosate<sup>3</sup> at rates of 870, 1,730, 3,470, and 6,930 g ha<sup>-1</sup>; pyriithiobac<sup>4</sup> at rates of 70, 140, 280, and 420 g ha<sup>-1</sup>; and glyphosate plus pyriithiobac at rates of 870 + 70, 1,730 + 140, 3,470 + 280, and 6,930 + 420 g ha<sup>-1</sup>. A nonionic surfactant (0.25% v:v) was included when pyriithiobac was applied singly. A treatment with no pyriithiobac or glyphosate control was included for comparison. The treatments were arranged in a randomized complete block design (RCBD) with four replicates. All herbicides were applied to 5- to 10-cm tall suspected GLY/ALS-R Palmer amaranth (May 12 and 25 in 2007 and 2008, respectively) at densities of 4 to 10 plants m<sup>-2</sup> with a CO<sub>2</sub>-pressurized sprayer equipped with flat fan nozzles delivering 168 L ha<sup>-1</sup> at 165 kPa. All plots received two applications of S-metolachlor<sup>5</sup> year<sup>-1</sup> at 1,392 g ha<sup>-1</sup> (June 3 and 18, 2007, and May 23 and June 7, 2008) to prevent additional Palmer amaranth emergence.

Visual estimates of control were obtained 1, 3, 5, and 8 WAT using a scale of 0 (no control) to 100% (complete control) (Frans et al. 1986). Visual control ratings for each observation date were analyzed separately using PROC MIXED in SAS.<sup>6</sup> Year and replicate within year were considered random effects. Contrasts were used to make relevant treatment comparisons.

**Greenhouse Experiment.** Two runs of the experiment were initiated in April and November of 2008. The greenhouse was maintained between 30 and 36 C with natural light supplemented for 12 hr each day with metal halide lamps (400 μ E m<sup>-2</sup> s<sup>-1</sup>). Seeds of S, GLY-R, ALS-R, and the putative GLY/ALS-R biotypes were planted, separately, into 15-cm<sup>3</sup> pots containing a local sandy loam topsoil. The S seeds, which were susceptible to both glyphosate and pyriithiobac, were collected from a population at the University of Georgia's

Attapulgus Research and Education Center in Attapulgus, GA. GLY-R and GLY/ALS-R seeds were collected from plants at the Macon County field site; the GLY-R seeds were collected in 2005, prior to reports of pyriithiobac resistance (Culpepper et al. 2006). The ALS-R seeds were collected from a mixed population that had been previously screened and confirmed for ALS-resistance and advanced in the greenhouse (Wise et al. 2009). Seedlings were thinned to one plant per pot and were watered and fertilized as needed.

Seedlings, 10 to 15 cm in height, were treated with glyphosate and pyriithiobac to establish dose-response curves for each biotype-herbicide combination. Glyphosate was applied POST to the S and ALS-R biotypes at rates of 39, 79, 118, 197, 236, 315, and 3,151 g ha<sup>-1</sup>; GLY-R and GLY/ALS-R plants were treated with glyphosate applied POST at 39, 315, 630, 945, 1,260, 1,576, 2,362, and 3,151 g ha<sup>-1</sup>. The S and GLY-R biotypes were treated with pyriithiobac at 2, 5, 9, 18, 35, 70, 140, 420, and 1,680 g ha<sup>-1</sup>; pyriithiobac was applied to the ALS-R and GLY/ALS-R plants at rates of 2, 70, 140, 420, 560, 1,120, and 1,680 g ha<sup>-1</sup>. A nonionic surfactant (0.25% v:v) was included when pyriithiobac was applied alone. A nontreated check was included for comparison. The differential rate structures were used to account for the variability in herbicide sensitivity among biotypes. All herbicides were applied in a custom-built spray chamber equipped with an even flat fan nozzle delivering 168 L ha<sup>-1</sup> at 165 kPa. Treatments were arranged in a RCBD with four replicates.

Estimates of visual injury were obtained 3 to 4 WAT using a scale of 0 (no injury) to 100% (plant death). Plants were then clipped at the soil line and above-ground fresh weights measured. Injury and shoot fresh weight, expressed as a percent of the nontreated control, were regressed against the log<sub>10</sub> of herbicide rate as described by Seefeldt et al. (1995) using PROC NLIN in SAS.

## Results and Discussion

**Field Experiment.** Glyphosate and pyriithiobac applied alone at rates of 870 and 70 g ha<sup>-1</sup>, respectively, failed to provide more than 31% control of Palmer amaranth 1 to 8 WAT

Table 2. Parameter estimates and associated model statistics for the log-logistic dose response curves for plant injury ratings and relative fresh weights for Palmer amaranth biotypes in response to glyphosate and pyriithiobac.

Biotype <sup>a</sup>	Model parameters <sup>b</sup>				Pseudo- $R^{2c}$	RF <sup>d</sup>
	$C$	$D$	$b$	Rate ( $I_{50}/FW_{50}$ )		
Glyphosate						
Injury						
S	2.9	102.5	−2.6	91.2	0.96	1.0
ALS-R	6.0	104.5	−2.2	102.8	0.95	1.1
GLY-R	−0.6	90.1	−4.3	1,449.6	0.96	15.9
GLY/ALS-R	0.9	102.1	−3.8	1,101.5	0.98	12.1
Fresh weight						
S	−0.6	98.3	5.4	79.2	0.86	1.0
ALS-R	−3.6	97.9	4.2	80.6	0.89	1.0
GLY-R	13.5	99.3	12.4	1,439.6	0.64	18.2
GLY/ALS-R	−0.3	102.4	9.1	1,097.9	0.70	13.9
Pyriithiobac						
Injury						
S	−0.5	98.1	−2.9	3.5	0.97	1.0
ALS-R	−1.4	156.0	−2.6	1,415.3	0.88	404.3
GLY-R	−1.6	96.0	−3.1	22.6	0.93	6.5
GLY/ALS-R	−0.3	76.2	−5.8	528.8	0.94	151.0
Fresh weight						
S	6.0	100.1	3.0	2.0	0.86	1.0
ALS-R	9.4	98.4	4.7	606.9	0.51	303.5
GLY-R	8.2	101.9	3.3	21.0	0.66	10.5
GLY/ALS-R	−19.2	99.7	3.3	1,125.7	0.55	562.9

<sup>a</sup> Abbreviations: S, susceptible; ALS-R, acetolactate synthase inhibiting herbicide-resistant; GLY-R, glyphosate-resistant; and GLY/ALS-R, glyphosate/acetolactate synthase inhibiting herbicide-resistant.

<sup>b</sup> The log-logistic dose response model is defined as  $y = C + \frac{D - C}{1 + (x/rate_{50})^b}$  where  $C$  = lower limit,  $D$  = upper limit,  $b$  = slope, and  $rate_{50}$  = the herbicide rate required to achieve 50% response.

<sup>c</sup> Pseudo- $R^2 = 1 - SS(Residual)/SS(Total_{Corrected})$ .

<sup>d</sup> The resistance factor is defined as  $RF = rate_{50}(\text{resistant})/rate_{50}(\text{susceptible})$ .

(Table 1). As herbicide application rates increased, the level of Palmer control also increased, although no treatment provided commercially acceptable control (Table 1). Glyphosate at 1,730 g ha<sup>-1</sup> provided between 19 and 34% control of the suspected GLY/ALS-R Palmer amaranth biotype 1 to 8 WAT; glyphosate at 3,470 and 6,930 g ha<sup>-1</sup> provided no more than 68 and 89% control of Palmer amaranth, respectively, for any observation date (Table 1). Pyriithiobac applied at 140 g ha<sup>-1</sup> controlled Palmer amaranth 39, 39, 32, and 17% at 1, 3, 5, and 8 WAT, respectively (Table 1). Palmer amaranth control 1 to 8 WAT ranged from 28 to 65% when pyriithiobac was applied at

280 and 420 g ha<sup>-1</sup> (Table 1). When applied as a tank mixture, glyphosate at 870 g ha<sup>-1</sup> plus pyriithiobac at 70 g ha<sup>-1</sup> controlled Palmer amaranth 16 to 41% 1 to 8 WAT (Table 1). When glyphosate plus pyriithiobac was applied at 1,730 + 140 g ha<sup>-1</sup>, Palmer amaranth control was 57, 70, 55, and 48% at 1, 3, 5, and 8 WAT, respectively (Table 1). At rates of 3,470 + 280 and 6,930 + 420 g ha<sup>-1</sup>, control of the putative GLY/ALS-R population ranged from 71 to 95% (Table 1).

**Greenhouse Experiment.** Changes in visual injury ratings and relative fresh weights for the S, GLY-R, ALS-R, and the

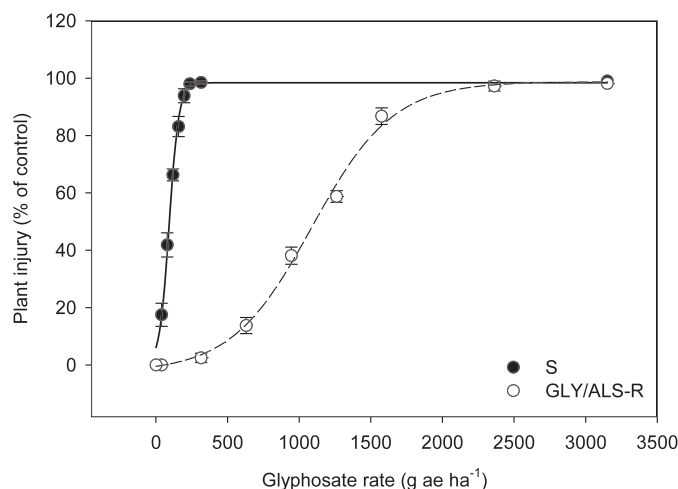


Figure 1. Plant injury for the susceptible (S) and glyphosate/acetolactate synthase inhibiting herbicide-resistant (GLY/ALS-R) Palmer amaranth biotypes in response to glyphosate at 3 to 4 wk after treatment (WAT).

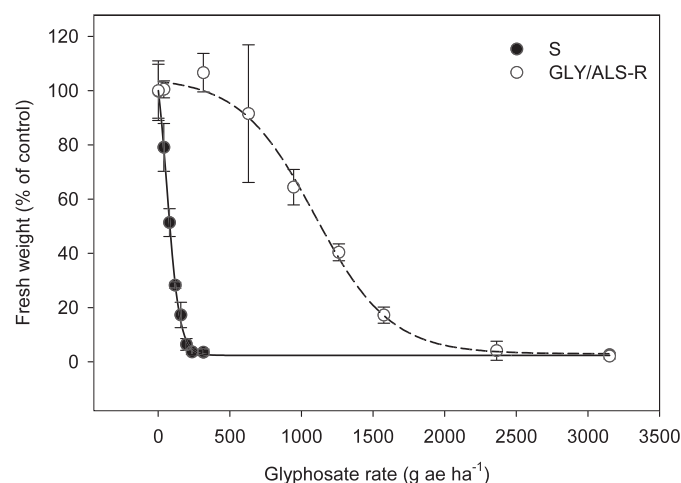


Figure 2. Fresh weight reductions for the susceptible (S) and glyphosate/acetolactate synthase inhibiting herbicide-resistant (GLY/ALS-R) Palmer amaranth biotypes in response to glyphosate at 3 to 4 wk after treatment (WAT).



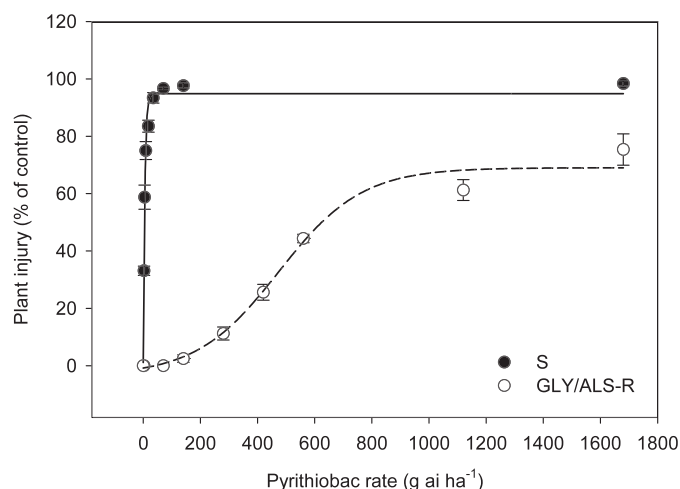


Figure 3. Plant injury for the susceptible (S) and glyphosate/acetolactate synthase inhibiting herbicide-resistant (GLY/ALS-R) Palmer amaranth biotypes in response to pyrithiobac at 3 to 4 wk after treatment (WAT).

putative GLY/ALS-R biotypes in response to increasing rates of glyphosate and pyrithiobac were described with log-logistic dose-response curves (Table 2). The estimated rates of glyphosate required to produce 50% injury ( $I_{50}$ ) 3 to 4 WAT for the S and ALS-R biotypes were 91 and 103 g ha<sup>-1</sup>, respectively. The predicted  $I_{50}$  values for the GLY-R and GLY/ALS-R biotypes were 1,450 and 1,102 g ha<sup>-1</sup>, respectively, which were 16 and 12 times greater than the S biotype. Estimated glyphosate rates of 1,440 and 1,098 g ha<sup>-1</sup> were needed to reduce GLY-R and GLY/ALS-R fresh weights by 50% ( $FW_{50}$ ); these values are 18 and 14 times greater than the predicted  $FW_{50}$  value (79 g ha<sup>-1</sup>) for the S biotype. Injury ratings and fresh weight reductions of  $\geq 90\%$  were achieved at glyphosate rates of 197 g ha<sup>-1</sup> and 2,363 g ha<sup>-1</sup> for the S and GLY/ALS-R biotypes, respectively (Figures 1 and 2).

In a previous greenhouse study, the GLY-R Palmer amaranth biotype from Macon County was determined to have a glyphosate  $I_{50}$  of 1,200 g ha<sup>-1</sup>, which was eight times greater than that of the S biotype ( $I_{50}$  = 150 g ha<sup>-1</sup>); glyphosate applied at 12 times the recommended label rate failed to control the same population in the field (Culpepper et al. 2006). Several GLY-R Palmer amaranth biotypes in North Carolina had  $I_{50}$  values for glyphosate between 180 and 360 g ha<sup>-1</sup>, which were two to four times greater than the local S biotype ( $I_{50}$  = 89 g ha<sup>-1</sup>); the most resistant biotype had a glyphosate  $I_{50}$  of 1,960 g ha<sup>-1</sup>, which was 22 times the S check (York 2007). A GLY-R Palmer amaranth biotype from Arkansas had an  $I_{50}$  value of 2,800 g ha<sup>-1</sup> compared to 35 g ha<sup>-1</sup> for the S (Norsworthy et al. 2008).

The estimated rate of pyrithiobac required to cause 50% injury 3 to 4 WAT for the S biotype was 4 g ha<sup>-1</sup> (Table 2). The predicted  $I_{50}$  values for the ALS-R and GLY/ALS-R biotypes were 1,415 and 529 g ha<sup>-1</sup>, respectively, which are 404 and 151 times greater than that of the S biotype. The predicted  $FW_{50}$  values for the ALS-R and GLY/ALS-R biotypes are 304 and 563 times greater than the predicted  $FW_{50}$  value (2 g ha<sup>-1</sup>) for the S biotype. The predicted  $I_{50}$  (23 g ha<sup>-1</sup>) and  $FW_{50}$  (21 g ha<sup>-1</sup>) values for the GLY-R biotype are 7 and 11 times greater, respectively, than the  $I_{50}$  and  $FW_{50}$  values for the S biotype; this may indicate that a low level of pyrithiobac-resistance exists in this population. This is not entirely unexpected as the seed for the GLY-R population were collected

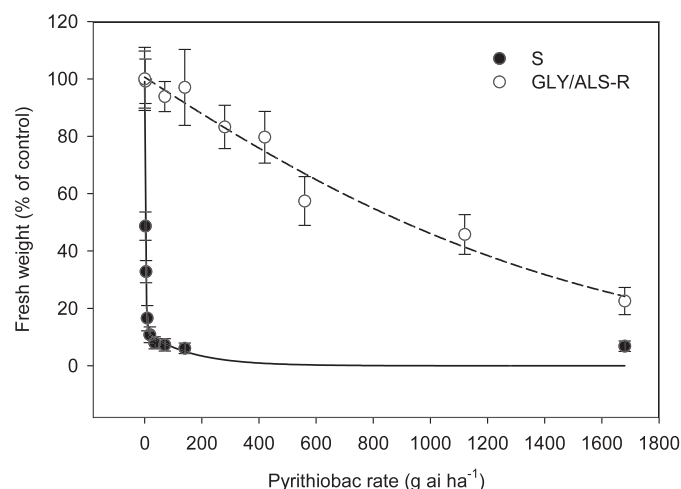


Figure 4. Fresh weight reductions for the susceptible (S) and glyphosate/acetolactate synthase inhibiting herbicide-resistant (GLY/ALS-R) Palmer amaranth biotypes in response to pyrithiobac 3 to 4 wk after treatment (WAT).

from the progenitors of the GLY/ALS-R biotype. Maximum injury ratings of 77% and fresh weight reductions of 78% were achieved for the GLY/ALS-R population when pyrithiobac was applied at 1,680 g ha<sup>-1</sup> (Figures 3 and 4).

Wise et al (2009) evaluated 10 Palmer amaranth accessions using dose-response curves to determine their level of resistance to imazapic. Eight accessions had  $FW_{50}$  values that were 5 to 199 times greater than the susceptible check ( $FW_{50}$  = 0.9 g ha<sup>-1</sup>); the remaining two accessions displayed extremely high levels of resistance to imazapic and had  $FW_{50}$  values > 1,400 g ha<sup>-1</sup> (Wise et al. 2009). In Arkansas, Palmer amaranth plants with intermediate levels of resistance to imazaquin were 14 to 16 times more tolerant of the herbicide than the susceptible check; highly resistant populations possessed 141 to 196 times greater tolerance (Burgos et al. 2001). Palmer amaranth populations from Kansas were not injured by 560 g ha<sup>-1</sup> of imazethapyr, which is eight times the field-use rate (Gaeddert et al. 1997; Horak and Peterson 1995).

This is one of the first reports of multiple resistance to both glyphosate and pyrithiobac in Palmer amaranth. Bond et al. (2010) also reported resistance pyrithiobac in GLY-R Palmer amaranth in Mississippi. Resistance to different herbicide modes of action has been previously observed in the genus *Amaranthus*. Horak and Peterson (1995) reported on the occurrence of resistance to imazethapyr and thifensulfuron in both Palmer amaranth and common waterhemp (*Amaranthus rudis* Sauer). Common waterhemp has also displayed multiple resistance to several ALS-inhibiting herbicides plus atrazine (Foes et al. 1998) and glyphosate, ALS- and protoporphyrinogen oxidase-inhibiting herbicides (Legleiter and Bradley 2008). Multiple resistance to imazethapyr and atrazine has been reported in Powell amaranth (*Amaranthus powellii* S. Wats) (Diebold et al. 2003).

Herbicide resistance in a weed population can either develop through genetic mutation or be acquired through pollen- and seed-mediated gene flow (Jasieniuk et al. 1996). Palmer amaranth seeds are likely being dispersed in water, with the movement of animals, and through agricultural management practices such as plowing, mowing, and harvesting (Costea et al. 2004, 2005; Menges 1987). Palmer amaranth is dioecious, and there is evidence to indicate that resistance traits can be disseminated through pollen

dispersal (Franssen et al. 2001; Sosnoskie et al. 2009; Wetzel et al. 1999). As multiple herbicide resistance within Palmer amaranth populations becomes more common, a grower's ability to be economically sustainable is threatened.

Glyphosate-resistant Palmer amaranth that escapes at-plant herbicide applications can only be controlled in glyphosate-resistant or conventional cotton using early-season POST applications of pyriithiobac. Palmer amaranth with resistance to both glyphosate and pyriithiobac cannot be managed using POST-applied herbicides in either glyphosate-resistant or nontransgenic cotton. The only POST herbicide available for growers to control emerged GLY/ALS-R Palmer amaranth is glufosinate; however, applications must be extremely timely and only cultivars tolerant to glufosinate can be planted (Culpepper et al. 2008; Marshall 2009). Also of enormous concern is the over dependence and tremendous selection pressure currently being placed on glufosinate for the control of Palmer amaranth. Growers with populations exhibiting multiple resistance will need to rely on nonchemical alternate control methods, such as tillage or cover crop mulches, to manage Palmer amaranth. Growers should ensure that Palmer amaranth plants in a production field do not reach reproductive maturity to prevent the local and long-distance spread of the resistance traits by seed and pollen.

## Sources of Materials

- <sup>1</sup> DP 145 B2RF and DP 555 BRR, Monsanto Company, 800 North Lindberg Ave., St. Louis, MO 63167.
- <sup>2</sup> Prowl H<sub>2</sub>O, BASF Corporation, 26 Davis Dr., Research Triangle Park, NC 27709.
- <sup>3</sup> Roundup WeatherMax, Monsanto Company, 800 North Lindberg Ave., St. Louis, MO 63167.
- <sup>4</sup> Staple LX, E. I. du Pont and Nemours and Company, Wilmington, DE 19898.
- <sup>5</sup> Parrlay, Monsanto Company, 800 North Lindberg Ave., St. Louis, MO 63167.
- <sup>6</sup> PROC MIXED, SAS Institute Inc., 100 SAS Campus Dr., Cary, NC 27513.

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