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Multiple Resistance to Glyphosate and Pyrithiobac in Palmer Amaranth (*Amaranthus palmeri*) from Mississippi and Response to Flumiclorac

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Greenhouse and laboratory studies were conducted to confirm and quantify glyphosate resistance, quantify pyrithiobac resistance, and investigate interaction between flumiclorac and glyphosate mixtures on control of Palmer amaranth from Mississippi. The GR_{50} (herbicide dose required to cause a 50% reduction in plant growth) values for two glyphosate-resistant biotypes, C1B1 and T4B1, and a glyphosate-susceptible (GS) biotype were 1.52, 1.3, and 0.09 kg ae ha^{-1} glyphosate, respectively. This indicated that the C1B1 and T4B1 biotypes were 17- and 14-fold resistant to glyphosate, respectively, compared with the GS biotype. The C1B1 and T4B1 biotypes were also resistant to pyrithiobac, an acetolactate synthase (ALS) inhibitor, with GR_{50} values of 0.06 and 0.07 kg ai ha^{-1}, respectively. This indicated that the C1B1 and T4B1 biotypes were 7- and 8-fold, respectively, more resistant to pyrithiobac compared with the GS biotype, which had a GR_{50} value of 0.009 kg ha^{-1}. Flumiclorac was antagonistic to glyphosate by reducing glyphosate translocation. The C1B1 and T4B1 absorbed less glyphosate 48 h after treatment (HAT) compared with the GS biotype. The majority of the translocated glyphosate accumulated in the shoot above the treated leaf (that contains the apical meristem) in the GS biotype and in the shoot below the treated leaf in the resistant biotypes, C1B1 and T4B1, by 48 HAT. The C1B1 biotype accumulated negligible shikimate levels, whereas the T4B1 and GS biotypes recorded elevated levels of shikimate. Metabolism of glyphosate to aminomethylphosphonic acid was not detected in either of the resistant biotypes or the susceptible GS biotype. The above results confirm multiple resistance to glyphosate and pyrithiobac in Palmer amaranth biotypes from Mississippi and indicate that resistance to glyphosate is partly due to reduced absorption and translocation of glyphosate.

Nomenclature: Flumiclorac; glyphosate; pyrithiobac; Palmer amaranth, *Amaranthus palmeri* S. Wats. AMAPA.

Key words: Absorption, AMPA, antagonism, shikimate, translocation.

Glyphosate is a nonselective, broad-spectrum, systemic, POST herbicide that has been used extensively throughout the world over the past four decades. It inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) (EC 2.5.1.19), thus inhibiting biosynthesis of aromatic amino acids. This leads to several metabolic disturbances, including inhibition of protein and secondary product biosynthesis (Franz et al. 1997) and deregulation of the shikimate pathway, leading to general metabolic disruption (Duke et al. 2003). Since its commercialization in 1974, glyphosate has been used extensively in both crop and noncrop lands. Because of its lack of selectivity, glyphosate use was initially limited to preplant, postdirected, preharvest, and postharvest applications for weed control. With the introduction of glyphosate-resistant crops in the mid-1990s, glyphosate is now widely used for weed control in glyphosate-resistant crops without concern for crop injury. Glyphosate-resistant crops are currently grown in several countries, with phenomenal adoption in the United States, Canada, Argentina, and Brazil. The widespread adoption of glyphosate-resistant crops has not only caused weed species shifts in these crops but has also resulted in evolution of glyphosate-resistant weed populations.

To date, 21 weed species are reported to be resistant to glyphosate worldwide (Heap 2011). Among these weeds, the first evidence of evolved resistance to glyphosate was reported in rigid ryegrass (*Lolium rigidum* Gaud.). (Pratley et al. 1996, 1999). In 2006, glyphosate resistance was documented in Palmer amaranth for the first time (Culpepper et al. 2006). During the past 5 yr, glyphosate-resistant Palmer amaranth populations have been reported from Arkansas (Norsworthy et al. 2008a,b), Tennessee (Steckel et al. 2008), and nine other states (Heap 2011) in the United States.

Research is being conducted extensively at various institutions to devise effective alternative strategies for management of glyphosate-resistant weed populations. One such approach is the use of residual herbicide programs. Another strategy is tank mixing herbicides, possessing mechanism(s) of action different from that of glyphosate, with glyphosate for applications as preplant or as selective in-season treatments to manage glyphosate-resistant weeds as well as to broaden the spectrum of weeds controlled. It has been reported that 87 to 95% control of 10-cm-tall Palmer amaranth was achieved when treated with glyphosate plus flumiclorac, compared with only 60 to 70% control with glyphosate alone (Larry Steckel, unpublished data). Flumiclorac is labeled for Palmer amaranth suppression (Anonymous 2006). The mechanism of action of flumiclorac is inhibition of protoporphyrinogen oxidase (PPO), leading to bleaching and wilting of leaf tissue. Herbicides such as flumiclorac that cause disruption of cell membranes inhibit absorption and translocation of other herbicides when applied in combination. The effect of flumiclorac on the absorption, translocation, and distribution of a systemic herbicide such as glyphosate is not clear.

In Mississippi, failure of glyphosate in controlling Palmer amaranth was reported several times in 2007. During the summer and fall months of 2007, seed samples from several

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Palmer amaranth populations suspected to be resistant to glyphosate were collected. Therefore, the objectives of this research were (1) to confirm whether Palmer amaranth populations from Mississippi are resistant to glyphosate and to quantify the magnitude of glyphosate resistance, (2) to screen alternative herbicide options for controlling Palmer amaranth and quantify resistance to pyrithiobac, and (3) to investigate interactions between flumiclorac and glyphosate mixtures on Palmer amaranth control by determining patterns of absorption and translocation of glyphosate applied alone and in combination with flumiclorac in Palmer amaranth.

Materials and Methods

Seed Collection, Storage, Germination, Planting, Growth, and Herbicide Treatment. In the summer and fall of 2007, seed from putatively glyphosate-resistant Palmer amaranth plants, was collected from row crop areas across the northwestern region of Mississippi (Mississippi Delta counties). These plants survived at least one in-season application of glyphosate at 0.84 kg ae ha^{-1}. Thirty-five populations were collected, with each population defined as a group of 10 to 20 randomly selected female (seed-bearing) plants from an individual field. Each population was from a different field. Palmer amaranth inflorescence spikes containing seeds were air-dried in a greenhouse (25/20 C day/night, 12-h photoperiod under natural sunlight conditions) for 7 d, cleaned, and stored at 2 to 8 C until further use. Germination of seeds, transplanting of seedlings, growth of plants, and all experiments were conducted under the following growing conditions unless otherwise described. Seeds were planted at 1-cm depth in 50-cm by 20-cm by 6-cm plastic trays with holes containing a commercial potting mix [formulated Canadian sphagnum peat moss, coarse perlite, bark ash, starter nutrient charge (with gypsum) and slow release nitrogen and dolomitic limestone] (Metro-Mix 360, Sun Gro Horticulture, Bellevue, WA 98008). Two weeks after emergence, Palmer amaranth plants were transplanted into 6-cm by 6-cm by 6-cm pots containing Metro-Mix 360. Plants were fertilized once with a nutrient solution (Miracle-Gro, The Scotts Company LLC, Marysville, OH 43041) containing 200 mg/L each of N, P_2O_5, and K_2O one wk after transplanting and subirrigated as needed. All herbicide treatments were applied with a moving nozzle sprayer equipped with 8002E nozzles (Spraying Systems Co., Wheaton, IL 60189) delivering 140 L ha^{-1} at 280 kPa, with all POST treatments made on Palmer amaranth plants that were 10-cm tall and at the four- to six-leaf stage. Procedures of planting and PRE application of herbicide treatment are described in a following section. A known susceptible (to all herbicide chemistries labeled for Palmer amaranth control) accession from Washington County, Mississippi (Charles Bryson, personal communication), hereafter referred to as glyphosate-susceptible (GS), was included for comparison in all experiments. All studies were conducted during the months of January to March and September to November in 2008 and 2009.

Screening of Populations with a Discriminating Glyphosate Dose. In preliminary glyphosate (Roundup WeatherMAX®, Monsanto Company, St. Louis, MO 63167) resistance screening studies, several Palmer amaranth populations survived a sublethal 0.42 kg ha^{-1} rate (data not shown). In follow-up experiments, 12 populations (ranging from 13 to 270 plants per population) were considered to be resistant to glyphosate on the basis of percent control (visible estimate of injury on a scale of 0 [no injury] to 100 [complete death]), 10 to 50%, and mortality (percentage of plants surviving [evidence of shoot regrowth at time of evaluation] in relation to total number of plants treated), 1 to 45%, measured 2 wk after treatment (WAT) with glyphosate at 0.84 kg ha^{-1} (Table 1).

Development of Second-Generation Resistant Biotypes. Palmer amaranth is dioecious (i.e., male and female flowers develop on different plants). Within each of the 12 resistant populations (see previous section), a single (resistant) male plant and several female (resistant) plants were selected at the first identification of the sex of the plant after flowering. Additional plants (clones) from the selected male and female plants were generated by planting 5-cm-long, 2-mm-diam stem segments containing at least one axillary meristem. The lower end of each segment was dipped in a root hormone, 0.1% indole-3-butyric acid, (TakeRoot®, Shultz Company, Bridgeton, MO 63044) powder and planted in pots containing the potting mix mentioned before. The segments were allowed to grow at least 5 cm before transplanting to a larger 4-L pot. This procedure provided an assured supply of male plants for pollen availability and female plants for increasing seed stocks. Male and female plants from a given population were grown together in isolation from other populations in growth chambers (24/18 C day/night temperatures, 16-h photoperiod, 600 μmol m^{-2} s^{-1} light intensity) to avoid cross contamination by pollen from a male plant belonging to a different population. After ascertaining development of male and female flowers on respective plants, pollen from the male plants was physically spread on the female plant every morning over a period of 2 wk. Each female plant that produced viable seed was considered a unique biotype within a population, with all biotypes within the population having the same male parent. Mature seed spikes from the female plants were harvested and processed, and seed was stored as mentioned earlier. Palmer amaranth plants raised from the second-generation seed were confirmed to be resistant to glyphosate (data not shown) following previously described procedures. Twenty-two biotypes that were resistant to glyphosate were generated from the 12 first-generation resistant populations. Glyphosate dose–response experiments were conducted on all 22 second-generation resistant biotypes, but only the two most resistant biotypes were selected for subsequent research. Glyphosate dose–response experiments conducted on the most resistant biotypes, hereafter referred to as C1B1 and T4B1, are described below.

Glyphosate Dose Response. Palmer amaranth plants of C1B1 and T4B1 biotypes were treated with glyphosate at 0, 0.42, 0.84, 1.68, 3.36, and 6.72 kg ha^{-1}. No injury was observed from adjuvant overload at rates greater than 0.84 kg ha^{-1} by use of a commercial formulation of glyphosate (data not shown). Plants of the GS biotype were treated with glyphosate at 0, 0.0033, 0.013, 0.05, 0.21, and 0.84 kg ha^{-1}. GS plants treated with glyphosate at rates similar to the C1B1 and T4B1 biotypes were completely

Table 1. Percent control and mortality of putative resistant Palmer amaranth populations from Mississippi 2 wk after treatment with glyphosate at 0.84 kg ae ha^{-1}.

Population	County	Control[a]	Mortality[a]
		%	
C1	Coahoma	50	7
T1	Tunica	30	8
T2	Tunica	10	16
T3	Tunica	20	2
T4	Tunica	10	6
T5	Tunica	30	24
T6	Tunica	10	36
T7	Tunica	10	3
T8	Tunica	30	7
T9	Tunica	30	1
T10	Tunica	30	45
T11	Tunica	20	8
GS[b,c]	Washington	100	100

[a] Control indicates visible estimate of injury on a scale of 0 (no injury) to 100 (complete death) and mortality indicates percentage of plants surviving (evidence of shoot regrowth at time of evaluation) in relation to total number of plants treated.

[b] Abbreviation: GS, glyphosate-susceptible.

[c] The GS accession is considered a biotype and not a population to maintain consistency in terminology (a population is considered a random collection of individual plants of a species, and a biotype is defined as a plant selection that has a unique genotypic pedigree, as in the case of the second-generation glyphosate-resistant biotypes).

controlled (data not shown), making it impractical to generate a glyphosate dose–response curve. Aboveground fresh weight of treated Palmer amaranth plants was measured 3 WAT and expressed as a percentage of aboveground fresh weight of nontreated plants. There were four replications per treatment.

Alternative Herbicides Evaluation (POST). Several herbicides with a mechanism of action different from that of glyphosate were applied POST to C1B1, T4B1, and GS plants. The herbicide treatments included paraquat (Gramoxone Inteon®, Syngenta Crop Protection Inc., Greensboro, NC 27419) at 0.56 kg ai ha^{-1}, glufosinate (Ignite® 280 SL, Bayer CropScience LP, Research Triangle Park, NC 27709) at 0.58 kg ai ha^{-1}, fomesafen (Reflex®, Syngenta Crop Protection Inc.) at 0.42 kg ai ha^{-1}, trifloxysulfuron (Envoke®, Syngenta Crop Protection) at 0.13 kg ai ha^{-1}, pyrithiobac (Staple® LX, DuPont Crop Protection, Wilmington, DE 19898) at 0.11 kg ai ha^{-1}, and chlorimuron (Classic®, DuPont Crop Protection) at 0.013 kg ai ha^{-1}. A crop oil concentrate (COC, Agridex®, Helena Chemical Co., Collierville, TN 38017) at 1% (v/v) was added to each of the paraquat and fomesafen treatments. A nonionic surfactant (NIS, Induce®, Helena Chemical Co.) at 0.25% (v/v) was added to each of trifloxysulfuron, pyrithiobac, and chlorimuron treatments. A glyphosate treatment at 0.84 kg ha^{-1} was also included. Palmer amaranth control was estimated on a scale of 0 (no injury) to 100% (plant death) 3 WAT. There were three replications per treatment.

Alternative Herbicides Evaluation (PRE). Soil (Bosket sandy loam, fine–loamy, mixed, thermic Mollic Hapludalfs; pH 8.2, 0.5% organic matter, cation exchange capacity = 16.7 meq [100 g]$^{-1}$, 51.3% sand, 37.1% silt, 11.6% clay) was added to 50 by 25 by 6-cm plastic trays without holes to a depth of 4 cm. Two-hundred milligrams of Palmer amaranth seed (approximately 450 seeds) was planted in 25-cm-long rows at a depth of 0.5 cm and covered with additional soil. An individual tray containing three rows, one planted to C1B1, T4B1, and GS biotypes, was considered a single replication. After seed placement, herbicide solutions were applied as described before, and trays were returned to the greenhouse. Herbicide treatments included *S*-metolachlor (Dual Magnum II® [Syngenta Crop Protection] at 1.1 kg ai ha^{-1}, fomesafen at 0.28 kg ha^{-1}, pendimethalin [Prowl H_2O®, BASF Corporation, Research Triangle Park, NC 27709] at 1.0 kg ai ha^{-1}, and pyrithiobac at 0.06 kg ha^{-1}). Trays were then watered immediately after herbicide application to activate the herbicide and as needed thereafter. Palmer amaranth seedlings that emerged and remained herbicide injury–free were counted 2 and 4 WAT. Pyrithiobac-susceptible plants emerged but remained chlorotic and stunted at 4 WAT. Herbicide efficacy was measured as percent decrease in cumulative seedling emergence compared with a nontreated control. There were three replications per treatment.

Pyrithiobac (POST) Dose Response. Palmer amaranth plants of C1B1, T4B1, and GS biotypes were treated with pyrithiobac at 0, 0.055, 0.11, 0.22, 0.44, and 0.88 kg ha^{-1}. An NIS at 0.25% (v/v) was added to all herbicide treatments, except the 0 kg ha^{-1} rate. Palmer amaranth control was estimated 3 WAT. There were four replications per treatment.

Glyphosate–Flumiclorac Tank-Mix Combination. C1B1, T4B1, and GS Palmer amaranth plants were treated with glyphosate at 0.84 kg ha^{-1} and flumiclorac (Resource®, Valent U.S.A. Corporation, Walnut Creek, CA 94596) at 0.06 kg ai ha^{-1}applied alone and in combination. All herbicide treatments had ammonium sulfate (AMS) included at 2% (wt/wt). The potassium salt formulation of glyphosate (Anonymous 2009) recommends AMS under certain conditions, such as hard water or drought. The growing and treatment conditions in this study did not warrant the addition of AMS; however, the addition of AMS when tank mixing flumiclorac with glyphosate has been recommended (V. F. Carey, personal communication). Palmer amaranth control was estimated 3 WAT. Treatments were replicated three times.

^{14}C-Glyphosate Absorption and Translocation. Separate groups of C1B1, T4B1, and GS plants were treated with glyphosate and glyphosate plus flumiclorac similarly, as described in the glyphosate–flumiclorac tank-mix combination study, except that the third fully expanded leaf was covered with a clear plastic sleeve. This sleeve was removed immediately after herbicide treatment for subsequent application of solutions containing ^{14}C-glyphosate (^{14}C-methyl labeled with 2.0 GBq $mmol^{-1}$ specific activity, 99.5% radiochemical purity in an aqueous stock solution of 7.4 MBq ml^{-1}, American Radiolabeled Chemicals Inc., St. Louis, MO 63146). Two solutions containing ^{14}C-glyphosate were prepared. First, a solution containing glyphosate at a final concentration of 0.84 kg ha^{-1} in 140 L was prepared using ^{14}C-glyphosate, a commercial formulation of glyphosate, and distilled water. Second, a solution with a final concentration of glyphosate plus flumiclorac at 0.84 + 0.06 kg/ha was made using ^{14}C-glyphosate, commercial formulations of glyphosate and flumiclorac, and distilled

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water. AMS was also added to both solutions at 2% (wt/wt). AMS addition did not affect glyphosate efficacy on Palmer amaranth (data not shown) but was added to the "glyphosate-only" treatment to be consistent with the glyphosate plus flumiclorac treatment. A 10-µl volume of the respective treatment solution was applied to the adaxial surface of the third true leaf of 10-cm-tall Palmer amaranth plants in the form of 25 droplets with a microapplicator. Plants were harvested at 24 and 48 h after ^{14}C-glyphosate treatment (HAT) and divided into treated leaf, shoot above treated leaf (SATL), shoot below treated leaf (SBTL), and roots. The treated leaf was immersed in 10 ml of 10% methanol in a glass vial and gently shaken for 20 s to remove non-absorbed ^{14}C-glyphosate remaining on the leaf surface. The leaf wash was repeated with an additional 10 ml of 10% methanol. Two 1-ml aliquots of each leaf wash were mixed with 10 ml of scintillation cocktail (Ecolume, ICN, Costa Mesa, CA 92626). The plant parts were wrapped in a single layer of tissue paper (Kimwipes, Kimberly-Clark Corporation, Roswell, GA), placed in a glass vial, and oven dried at 60 C for 48 h. Oven-dried plant samples were combusted in a biological oxidizer (Packard Instruments Company, Downers Grove, IL 60515), and the evolved $^{14}CO_2$ was trapped in 10 ml of Carbosorb E (Packard BioScience Company, Meridian, CT 06450) and 10 ml of Permaflour E+ (Packard BioScience). Radioactivity from leaf washes and oxidations was quantified by liquid scintillation spectrometry. The sum of ^{14}C present in the two leaf washes and oxidized plant parts represented 99% recovery of applied ^{14}C-glyphosate. Absorption was calculated as the sum of the radioactivity measured in all plant parts and is expressed as percentage of recovered ^{14}C. Total radioactivity recovered in all plant parts except the treated leaf was designated translocated ^{14}C and expressed as percentage of absorbed. There were three replications per treatment.

Efficacy of Single-Leaf–Treated Glyphosate on Whole Plant. Palmer amaranth plants of C1B1, T4B1, and GS biotypes were treated with a herbicide solution containing glyphosate at 0.84 kg ha^{-1} (1× field rate) in 140 L of water. Ten droplets of glyphosate solution (10 µl) was placed on the adaxial surface of a third fully expanded leaf. Plants were harvested 3 WAT, and aboveground fresh weight was measured and expressed as a percentage of aboveground fresh weight of nontreated plants. There were three replications per treatment.

Shikimate Assay with Leaf Discs. Shikimate assay on Palmer amaranth biotypes was conducted following previously reported protocols (Shaner et al. 2005). Leaf discs (6 mm diam) were excised from leaves with a common hand-held single-hole paper punch. Twenty leaf discs were added to 20-mL glass vials containing 1 ml of 10 mM ammonium phosphate (pH 4.4) plus 0.1% (v/v) Tween 80 surfactant solution and various concentrations of glyphosate (0, 7.8, 31.2, 125, 500, and 1000 µM). Vials were then placed in a controlled environment chamber equipped with fluorescent and incandescent bulbs (400 µmol m^{-2} s^{-1}) for 16 h at 25 C. Immediately after the 16-h incubation period, vials were frozen. Soon thereafter, 250 µl of 1.25 N HCl was added to each vial, and vials were thawed at 60 C for 30 min. The leaf discs turned gray, indicating complete penetration of leaf by the acid. Shikimate was then determined spectrophotometrically (Synergy HT Microplate Reader, BioTek Instruments Inc., Winooski, VT 05404) following the procedure of Cromartie and Polge (2000). Vials were vortexed, and a 25-µl aliquot from the vial was added to an individual well of a microtiter plate containing 100 ml of a mixture of 0.25% (wt/v) periodic acid (Sigma Chemical Co., St. Louis, MO 63103) and 0.25% (wt/v) *m*-periodate (Sigma Chemical Co.). The microtiter plate was incubated at room temperature (25 C) for 90 min and followed by the addition of 100 µl of a mixture of 0.6 N sodium hydroxide and 0.22 M sodium sulfite. The absorbance of the solutions in the microtiter plate wells was measured at 380 nm within 30 min. Background absorbance was subtracted from readings of the glyphosate treatments. A standard curve was constructed on the basis of absorbance measurements of known concentrations of shikimate solutions to determine shikimate formed from glyphosate treatment (µg shikimate ml^{-1} solution).

Glyphosate Metabolism. Palmer amaranth samples from all three biotypes, C1B1, T4B1, and GS, were analyzed for metabolism of glyphosate to aminomethylphosphonic acid (AMPA). Plants of all three biotypes were treated with glyphosate at 0.42 kg ha^{-1} (0.5× field rate) as well as at their respective GR_{50} (see "Statistical Analysis") doses (C1B1, 1.52 kg ha^{-1}; T4B1, 1.3 kg ha^{-1}; GS, 0.09 kg ha^{-1}). At 1 WAT, plants were excised at the soil surface, washed with running water, rinsed with distilled water to remove glyphosate remaining on the leaf surface, and blotted dry with paper towels. All plant samples were air dried, ground, and analyzed for glyphosate and AMPA following extraction and derivatization procedures published previously (Reddy et al. 2008). One gram of ground plant tissue (from each treatment) was extracted with 10 ml of water in a 15-ml centrifuge tube, shaken, placed in a sonicating bath for 20 min, and then centrifuged (Sorvall RC 5C Plus; Kendro Laboratory Products, Asheville, NC 28787) at 47,000 Rpm, 20 C, for 20 min. Supernatant was removed. The tissue sample pellet was extracted a second time by adding 10 ml of water, and procedures were performed as in the first extraction. The volume of the combined supernatant was measured, and then 5 µl of 12.1 M HCl was added and shaken. Four milliliters was transferred to a 20-ml scintillation vial with a Teflon-lined cap, shaken with 4 ml of methylene chloride, and centrifuged (Savant speed vac model SVC 200, Savant Instruments Inc., Holbrook, NY 11741) for 10 min. A portion (1.8 ml) of the water layer was taken, and 200 µl of acidic modifier (16 g of KH_2PO_4, 160 ml of H_2O, 40 ml of methanol [MeOH], 13.4 mL of HCl) was added. One milliliter was loaded to a cation exchange resin column (AG 50W-X8, H+, Bio-Rad Laboratories, Hercules, CA 94547) previously equilibrated with two 5-ml portions of water. The sample was eluted to the level of column bed. CAX mobile phase (160 ml of H_2O, 40 ml of MeOH, 2.7 ml of HCl) (0.7 ml) was added, eluted, and discarded. Twelve milliliters of CAX mobile phase was again added to the column to elute the analytes. The eluate was collected in a 20-ml vial and evaporated to dryness using a Savant speed vac. To the dried sample was added 1.5 ml of CAX mobile phase, then the vial was placed in a sonicating bath for 30 min. A 20-µl aliquot was taken and added to 640 µl of a solution of 2,2,3,3,4,4,4-heptafluoro-1-butanol and trifluoroacetic anhydride (1 : 2) in

a chilled 4-ml vial. The mixture was allowed to equilibrate at room temperature for 10–15 min. The vial was transferred to a heating block at 90 C for 1 h and then allowed to cool to room temperature. The solvent was evaporated under a stream of nitrogen, and the residue was dissolved in 80 µl of ethyl acetate containing 0.2% citral; 50 µl was transferred to a gas chromatography (GC) vial and analyzed by GC–mass spectrometry (MS). This method afforded 90 and 86% recoveries of glyphosate and AMPA, respectively, on the basis of duplicate extraction experiments in which samples were fortified with 100-ng standards per gram of sample. Analysis of glyphosate and AMPA by GC-MS (Agilent 6890 series GC coupled to a JEOL GCMateII mass spectrometer) was performed using a DB-5 capillary column (J&W Scientific Inc., Folsom, CA 95630), 30 m long by 0.25-mm i.d. by 0.25-µm film. The MS detector was a magnetic sector; spectra were acquired in the positive, low-resolution, selected ion monitoring mode. The injection port, GC interface, and ionization chamber were maintained at 260, 230, and 120 C, respectively. The carrier gas was ultrahigh-purity helium at a 1 ml min^{-1} flow rate. The sample injection volume was 1 µl. Glyphosate and AMPA in the samples were quantitated from a calibration curve of the respective derivatized standards. For the analysis of glyphosate and AMPA, the temperature program was as follows: initial 70 C, held for 3.5 min, raised to 160 at a 30 C min^{-1} rate, raised to 270 at 70 C min^{-1}, raised to 310 at 35 C min^{-1}, and finally held at this temperature for 3 min. AMPA derivative was observed at 7:23 min (*m/z* 571, 502, 446, 372) and glyphosate derivative was observed at 7:59 min (*m/z* 611, 584, 460). The limit of detection (LOD) and limit of quantification (LOQ) for glyphosate were 19.9 and 160 pg on column (1 µl injection), respectively. The LOD and LOQ for AMPA were 3.71 and 11.2 pg on column (1 µl injection), respectively. Analysis was performed in duplicate. There were six replications per treatment.

Statistical Analysis. All experiments were conducted using a completely randomized design, and experiments were repeated once, except in the glyphosate metabolism study. All data were analyzed by ANOVA via the PROC GLM statement using SAS software (version 9.2, SAS Institute Inc., Cary, NC 27513). No significant experiment effect was observed in repeated experiments; therefore, data from experiments were pooled. Nonlinear regression analysis was applied to define the following two curves. (1) A sigmoidal log-logistic curve of the form

$$y = a/\{1 + \exp[-(x - x_0)/b]\} \quad [1]$$

related the effect of glyphosate dose (x) on Palmer amaranth growth (y), where a is an asymptote, x_0 is the glyphosate dose resulting in a given measure of y, and b is the slope of the curve around x_0. (2) A single rectangular hyperbolic curve of the form

$$y = ax/(b + x) \quad [2]$$

described the effect of pyrithiobac dose (x) on Palmer amaranth control (y), where a is an asymptote and b is the slope of the curve. Equation parameters were computed using SigmaPlot (version 11.0, Systat Software Inc., San Jose, CA 95110). Treatment means within all the other experiments were separated using Fisher's Protected LSD test at P = 0.05.

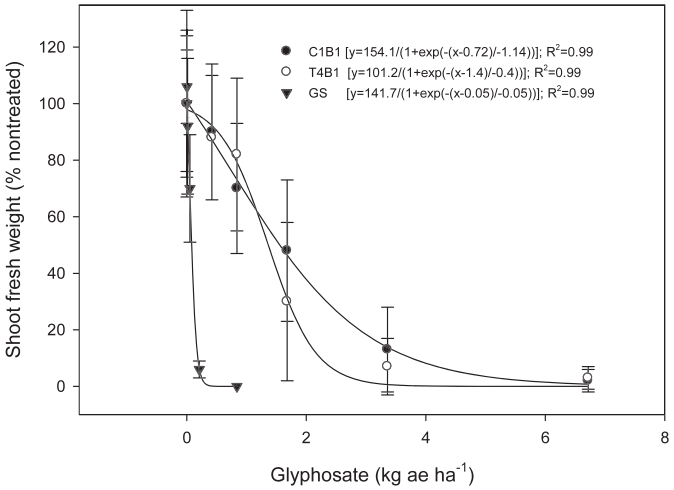

Figure 1. Glyphosate dose response on aboveground shoot growth of glyphosate-resistant (C1B1 and T4B1) and glyphosate-susceptible (GS) Palmer amaranth biotypes. GR_{50} (dose required to reduce plant growth by 50%) values for C1B1, T4B1, and GS biotypes were 1.52, 1.3, and 0.09 kg ae ha^{-1} glyphosate, respectively. Error bars represent standard deviation of treatment means.

Expected response in the glyphosate–flumiclorac tank-mix combination study was calculated using a previously described procedure (Colby 1967). If the observed response of the glyphosate and flumiclorac combination was significantly (LSD at 5% level of significance) lower or higher than the expected value, the combination was declared to be antagonistic or synergistic, respectively. Combinations were considered to be additive (no interaction) when the observed and expected responses were similar.

Results and Discussion

Glyphosate Dose Response. GR_{50} values, based on glyphosate dose–response results (Figure 1) for the C1B1, T4B1, and GS biotypes, from shoot fresh weight expressed as a percentage of nontreated control, were 1.52, 1.3, and 0.09 kg ha^{-1} glyphosate. This indicated that the C1B1 and T4B1 biotypes were 17- and 14-fold resistant to glyphosate, respectively, compared with the GS biotype. This level of glyphosate resistance is higher than that reported for a glyphosate-resistant Palmer amaranth biotype from Georgia that had a GR_{50} (also based on shoot fresh weight) of 0.56 kg ha^{-1} and was 6.2-fold more resistant than a susceptible biotype with a GR_{50} of 0.09 kg/ha (Culpepper et al. 2006). A common occurrence in both C1B1 and T4B1 biotypes was the stimulation of axillary growing points and continued growth at 0.84 and 1.68 kg ha^{-1} rates. Plants of these two biotypes remained physiologically active but were severely stunted at the 3.76 kg ha^{-1} rate and dead at the highest rate applied (6.72 kg ha^{-1}). The GS biotype was extremely sensitive to glyphosate, with complete death occurring at the 0.21 kg ha^{-1} rate (one fourth the 1× rate of 0.84 kg ha^{-1}).

Alternative Herbicides Evaluation (POST). All herbicides including glyphosate provided complete control (≥ 98%) of the GS biotype (Table 2). Glyphosate controlled C1B1 and T4B1 biotypes only 12 and 33%, respectively. Paraquat, glufosinate, and fomesafen effectively controlled (100%) the C1B1 and T4B1 biotypes by 3 WAT (Table 2). Thus, these three herbicides, representing different mechanisms of action,

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Table 2. Visual estimates of control of glyphosate-resistant (C1B1 and T4B1) and -susceptible (GS) Palmer amaranth biotypes at 3 wk after treatment with various POST-applied herbicides.

Herbicide[a]	Rate[b]	Biotype		
		GS[c]	C1B1	T4B1
	kg ha^{-1}	%		
Glyphosate	0.84	100	12	33
Paraquat	0.56	100	100	100
Glufosinate	0.59	100	100	100
Fomesafen	0.42	100	100	100
Trifloxysulfuron	0.13	100	72	77
Pyrithiobac	0.11	98	55	78
Chlorimuron	0.013	98	69	87
LSD (0.05)			13	

[a] A crop oil concentrate (Agridex) at 1% (v/v) was added to paraquat and fomesafen treatments. A nonionic surfactant (Induce) at 0.25% (v/v) was added to each of trifloxysulfuron, pyrithiobac, and chlorimuron.
[b] Glyphosate is expressed in kg ae ha^{-1} and all other herbicides in kg ai ha^{-1}.
[c] Abbreviation: GS, glyphosate-susceptible.

could be used as an alternative for managing glyphosate-resistant Palmer amaranth accompanied by responsible stewardship programs. Fomesafen belongs to the PPO-inhibiting herbicide family. PPO resistance has been documented in waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer], a species closely related to Palmer amaranth (Heap 2011). Control of Palmer amaranth biotypes C1B1 and T4B1 with the acetolactate synthase (ALS)–inhibiting herbicides trifloxysulfuron, pyrithiobac, and chlorimuron was variable, ranging from 55 to 78%, and less than the GS biotype except for T4B1 with chlorimuron (87% control) (Table 2). Norsworthy et al. (2008a) reported a similar range (63 to 84%) of control of glyphosate-resistant Palmer accessions from Arkansas with pyrithiobac and trifloxysulfuron. These results indicated potential resistance to one or more of the ALS-inhibiting herbicides in the C1B1 and T4B1 biotypes.

Alternative Herbicides Evaluation (PRE). Herbicide efficacy was calculated on the basis of cumulative emergence and survival at 4 WAT as a percent of a nontreated control. Nontreated control densities of Palmer amaranth plants for the C1B1, T4B1, and GS biotypes were 53, 45, and 62 plants per 25-cm row, respectively. Fomesafen, *S*-metolachlor, and pendimethalin provided excellent control (100%) of plants from all three biotypes (Table 3). Whereas control of the GS biotype was 90% with pyrithiobac, considered commercially effective, C1B1 and T4B1 were controlled only 52 and 64%, respectively (Table 3). This reduced level of control of C1B1 and T4B1 biotypes with pyrithiobac might indicate resistance in these biotypes, substantiating reduced control obtained with POST application of pyrithiobac.

Table 3. Palmer amaranth control with herbicides applied PRE under greenhouse conditions.[a]

Herbicide	Rate	Control (4 WAT)[b]		
		C1B1	T4B1	GS
	kg ai ha^{-1}	%		
Fomesafen	0.28	100	100	100
S-Metolachlor	1.1	100	100	100
Pendimethalin	1.0	100	100	100
Pyrithiobac	0.06	52	64	90
LSD (0.05)			6	

[a] Abbreviations: GS, glyphosate-susceptible; WAT, weeks after treatment.
[b] Control is calculated as % decrease in cumulative seedling emergence compared with a nontreated control. Data from respective nontreated treatments were not included in analysis of data. Palmer amaranth density in nontreated trays of C1B1, T4B1, and GS biotypes were 53, 45, and 62 plants (25-cm row)$^{-1}$, respectively.

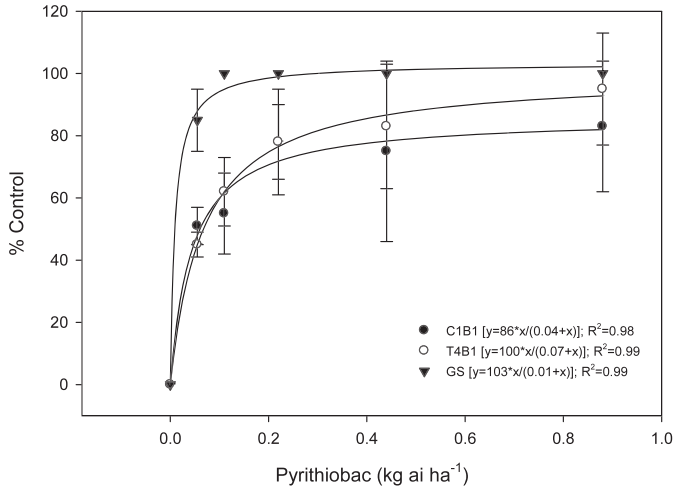

Figure 2. Pyrithiobac dose response on control of glyphosate-resistant (C1B1 and T4B1) and glyphosate-susceptible (GS) Palmer amaranth biotypes. GR_{50} (dose required to reduce plant growth by 50%) values for C1B1, T4B1, and GS biotypes were 0.06, 0.07, and 0.009 kg ai ha^{-1} pyrithiobac, respectively. Error bars represent standard deviation of treatment means.

Pyrithiobac (POST) Dose Response. Reduced control of the glyphosate-resistant biotypes, C1B1 and T4B1, with pyrithiobac applied POST or PRE prompted an investigation of pyrithiobac dose response on Palmer amaranth control (Figure 2). Pyrithiobac GR_{50} values of the C1B1, T4B1, and GS biotypes were 0.06, 0.07, and 0.009 kg ha^{-1}, respectively. This indicates that the C1B1 and T4B1 biotypes were 7- and 8-fold, respectively, more resistant to pyrithiobac compared with the GS biotype. Thus, the C1B1 and T4B1 biotypes have developed multiple resistance to glyphosate and pyrithiobac. A similar case of multiple resistance to glyphosate and pyrithiobac in a Palmer amaranth population from Georgia has recently been reported (Sosnoskie et al. 2011). The level of resistance to pyrithiobac in the Georgia biotypes was much higher than the Mississippi biotypes.

Glyphosate–Flumiclorac Tank-Mix Combination. The influence of tank mixing flumiclorac with glyphosate on Palmer amaranth control is summarized in Table 4. Control of C1B1, T4B1, and GS biotypes was 52, 65, and 100%, respectively, 3 WAT with glyphosate at 0.84 kg ha^{-1}. Control of Palmer amaranth with flumiclorac at 0.06 kg ha^{-1} ranged only from 22 to 43%. One of the reasons for this low level of control could be because flumiclorac killed the growing apex of treated Palmer amaranth plants rapidly and caused proliferation of lateral axillary meristems within the first week. By 3 WAT, plants had completely outgrown flumiclorac injury. Addition of flumiclorac to glyphosate caused an interactive effect on control of C1B1 and T4B1 in an antagonistic way, determined by calculating an expected magnitude of control, on the basis of Colby (1967) analysis, and comparing to the actual observed control (Table 4). Addition of flumiclorac to glyphosate provided only 47 to

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Table 4. Influence of flumiclorac on glyphosate efficacy on Palmer amaranth.

Biotype	Treatment	Rate[a]	Control[b] Observed	Control[b] Expected[c]
		kg ha^{-1}	%	
C1B1	Glyphosate	0.84	52	
	Flumiclorac	0.06	43	
	Glyphosate + flumiclorac	0.84 + 0.06	50	73*
T4B1	Glyphosate	0.84	65	
	Flumiclorac	0.06	22	
	Glyphosate + flumiclorac	0.84 + 0.06	47	73*
GS	Glyphosate	0.84	100	
	Flumiclorac	0.06	37	
	Glyphosate + flumiclorac	0.84 + 0.06	95	100
LSD (0.05)			15	

[a] Glyphosate is expressed in kg ae ha^{-1} and flumiclorac in kg ai ha^{-1}.
[b] Control indicates visible estimate of injury on a scale of 0 (no injury) to 100 (complete death).
[c] Expected values are calculated as described by Colby (1967); an asterisk adjacent to the expected control indicates antagonism, and no marking indicates an additive response. Interactions were considered significant if the difference between the observed and expected values exceeded the appropriate LSD value.

50% control of the glyphosate-resistant C1B1 and T4B1 biotypes, whereas control of the GS biotype decreased from 100 to 95% (an additive response). These greenhouse results are different from those of a field experiment (L. E. Steckel, unpublished data), in which control of similar sized, 10-cm-tall Palmer amaranth was 63% with glyphosate at 0.84 kg ha^{-1} and was enhanced to 90% with the addition of flumiclorac at 0.06 kg ha^{-1}. Our research was initiated to identify a potential alternative in flumiclorac for controlling Palmer amaranth including glyphosate-resistant accessions. The above results indicated an antagonistic interaction between glyphosate and flumiclorac. Koger et al. (2007) reported that MSMA antagonized glyphosate efficacy on Palmer amaranth. Glyphosate is a systemic translocatable herbicide, whereas flumiclorac is a contact-type herbicide. We hypothesized that the antagonism between glyphosate and flumiclorac could be due to reduced absorption, translocation of glyphosate in Palmer amaranth, or both caused by flumiclorac. This hypothesis was tested by measuring ^{14}C-glyphosate absorption and translocation in the presence of flumiclorac.

Flumiclorac Influence on ^{14}C-Glyphosate Absorption and Translocation. The effect of tank mixing flumiclorac on absorption and translocation of glyphosate is presented in Table 5. Because of a significant three-way interaction between the main effects of biotypes, herbicide treatment, and harvest time for ^{14}C-absorption and for visualization of patterns across all measured variables, all data are presented separated by main effects. The significance, or lack thereof, of main effects and of two-way and three-way interactions is shown by the corresponding LSD (0.05) values for each variable. Only the significant main effects and interactions are discussed below.

Biotype had a highly significant effect on ^{14}C-glyphosate absorption. Averaged across treatment and harvest time, ^{14}C-absorption in the C1B1, T4B1, and GS biotypes was 37, 28, and 42% of applied, respectively. This indicates that the T4B1 biotype absorbed less ^{14}C-glyphosate than the GS biotype. The C1B1 biotype absorbed similar amount of glyphosate as the other two biotypes. The three-way interaction between biotype, herbicide treatment, and harvest time significantly affected ^{14}C-absorption in Palmer amaranth plants. The lowest amount of ^{14}C-absorption (27 to 29% of applied) was recorded by the T4B1 biotype irrespective of treatment or harvest time. The highest amount of ^{14}C-

Table 5. Effect of flumiclorac on ^{14}C-glyphosate absorption, translocation, and distribution in Palmer amaranth.[a]

Biotype	Treatment[b]	Harvest time	Absorption	Translocation[c]	^{14}C-glyphosate distribution[d] TL	SATL	SBTL	Root
		h	% of applied		% of absorbed			
C1B1	Glyphosate	24	39	40	61	13	18	9
	Glyphosate + flumiclorac	24	36	21	79	4	9	8
T4B1	Glyphosate	24	27	37	63	8	14	15
	Glyphosate + flumiclorac	24	28	27	73	4	11	12
GS	Glyphosate	24	38	40	61	8	21	11
	Glyphosate + flumiclorac	24	44	26	74	6	11	10
C1B1	Glyphosate	48	35	56	43	18	25	13
	Glyphosate + flumiclorac	48	39	19	80	5	8	6
T4B1	Glyphosate	48	29	67	33	12	25	30
	Glyphosate + flumiclorac	48	28	29	72	5	11	13
GS	Glyphosate	48	50	46	53	23	18	15
	Glyphosate + flumiclorac	48	40	29	72	5	11	13
LSD (0.05)[e]								
Biotype			10	NS	NS	NS	NS	4
Treatment			NS	6	6	5	3	3
Harvest time			NS	6	6	NS	NS	3
Biotype × treatment			NS	NS	NS	NS	NS	NS
Biotype × harvest time			NS	NS	NS	NS	NS	NS
Treatment × harvest time			NS	9	9	NS	4	5
Biotype × treatment × harvest time			9	NS	NS	NS	NS	NS

[a] Abbreviations: GS, glyphosate susceptible, SATL, shoot above treated leaf; SBTL, shoot below treated leaf; TL, treated leaf.
[b] Glyphosate treatment contained glyphosate at a final concentration of 0.84 kg ae ha^{-1} in 140 L and glyphosate + flumiclorac treatment contained glyphosate at 0.84 kg ha^{-1} and flumiclorac at 0.06 kg ai ha^{-1}. Ammonium sulfate was added to both treatments at 2% (wt/wt).
[c] ^{14}C-glyphosate outside of treated leaf (SATL, SBTL, and root) was considered translocation.
[d] ^{14}C-glyphosate distribution throughout the plant is based on percentage of ^{14}C-absorbed.
[e] A number indicates significance at the 5% level of probability and NS indicates no significant influence of main effect or interaction.

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glyphosate was absorbed by the GS biotype with 50% of applied at 48 HAT when treated with glyphosate alone. The C1B1 biotype absorbed similar levels of glyphosate (35 to 39% of applied), irrespective of herbicide treatment or harvest time. Burke et al. (2007) recorded 57% (of applied) ^{14}C-glyphosate absorption in Palmer amaranth 48 HAT. Culpepper et al. (2006) reported similar levels of absorption of glyphosate between resistant and susceptible biotypes.

The effect of treatment, harvest time, and treatment by harvest time interaction on amount of ^{14}C-glyphosate that translocated out of the treated leaf was significant. Averaged across biotypes and harvest time, translocation of ^{14}C-glyphosate in glyphosate-only–treated plants was 48% of absorbed compared with only 25% of absorbed in glyphosate plus flumiclorac–treated plants. This clearly indicates that the contact activity of flumiclorac desiccated Palmer amaranth leaves, thereby, disrupting glyphosate translocation to other parts of the plant. MSMA reduced translocation of glyphosate by 7% in Palmer amaranth (Burke et al. 2007). Averaged across biotype and herbicide treatment, ^{14}C-glyphosate translocation was 32% of absorbed 24 HAT but increased to 41% of absorbed by 48 HAT. The amount of translocated ^{14}C-glyphosate, averaged across biotypes, was 38% of absorbed 24 HAT and increased to 58% of absorbed by 48 HAT when Palmer amaranth plants were treated with glyphosate alone. Conversely, translocated ^{14}C-glyphosate was only 25% of absorbed in plants treated with a combination of glyphosate and flumiclorac.

Distribution of ^{14}C-glyphosate in the treated leaf was affected by main and interaction effects similar to those of translocated ^{14}C-glyphosate described above. Because of a significant influence of treatment, amount of ^{14}C-glyphosate that remained in the treated leaves of glyphosate-treated plants was lower (52% of absorbed) compared with that in the treated leaves of glyphosate plus flumiclorac–treated plants (75% of absorbed). The amount of ^{14}C-glyphosate that remained in the treated leaf was greater at 24 HAT (68% of absorbed) compared with 48 HAT (59% of absorbed), averaged across treatments and biotypes. Furthermore, a significant interaction between treatment and harvest time resulted in higher ^{14}C-glyphosate (62% of absorbed) in the treated leaves of glyphosate-treated plants at 24 HAT compared with 42% of absorbed ^{14}C-glyphosate at 48 HAT, averaged across biotypes.

Distribution of ^{14}C-glyphosate in the SATL was affected by herbicide treatment. Averaged across biotypes and harvest time, ^{14}C-glyphosate was 14% of absorbed in glyphosate-treated plants compared with only 5% of absorbed in glyphosate plus flumiclorac–treated plants. Similarly, ^{14}C-glyphosate in SBTLs was affected by the herbicide treatment. Only 10% of absorbed ^{14}C-glyphosate remained in the SBTL of glyphosate plus flumiclorac–treated plants compared with twice as much (20% of absorbed) ^{14}C-glyphosate in SBTL of glyphosate-only–treated plants, averaged across biotype and harvest time. A mild herbicide treatment and harvest time interaction occurred affecting ^{14}C-glyphosate levels in SBTLs. Averaged across biotypes, ^{14}C-glyphosate in SBTLs was 18% of absorbed in glyphosate-treated plants 24 HAT compared with 23% of recovered at 48 HAT. On the other hand, a similar amount of ^{14}C-glyphosate (10% of absorbed) translocated to SBTLs in glyphosate plus flumiclorac–treated plants both at 24 and 48 HAT.

All three main effects, biotype, herbicide treatment, and harvest time, and the two-way treatment by harvest time interaction significantly affected the distribution of ^{14}C-glyphosate in the root. For example, averaged across treatment and harvest time, the T4B1 biotype accumulated more ^{14}C-glyphosate in the roots (17% of absorbed) than either C1B1 (9% of absorbed) or GS (12% of absorbed) biotypes. Averaged across biotypes and harvest time, roots of the glyphosate-treated plants had more ^{14}C-glyphosate compared with roots of the glyphosate plus flumiclorac–treated plants (15 vs. 10% of absorbed). The amount of ^{14}C-glyphosate in the roots was less (11% of absorbed) at 24 HAT compared with levels at 48 HAT (15% of absorbed), averaged across biotype and treatment. Levels of ^{14}C-glyphosate were 12% of absorbed at 24 HAT compared with 19% of absorbed at 48 HAT in glyphosate-treated plants, whereas glyphosate levels were same (10 to 11% of absorbed) at 24 and 48 HAT, averaged across biotypes.

Role of ^{14}C-Glyphosate Absorption, Translocation, and Distribution in Glyphosate Resistance Mechanism. This section is limited to discussing "glyphosate-only" treatments (Table 5) by numerically comparing ^{14}C-glyphosate absorption and translocation levels and distribution patterns in glyphosate-resistant and susceptible Palmer amaranth biotypes. The T4B1 biotype absorbed less ^{14}C-glyphosate (29% of applied) compared with the C1B1 (39% of applied) and the GS (38% of applied) biotypes at 24 HAT. By 48 HAT, both T4B1 (29% of applied) and C1B1 (35% of applied) biotypes absorbed less ^{14}C-glyphosate compared with the GS biotype (50% of applied). The amount of radioactivity that translocated out of the treated leaf was somewhat similar between all three biotypes (37 to 40% of absorbed) at 24 HAT but increased in the resistant C1B1 (56% of absorbed) and T4B1 (67% of absorbed) biotypes compared with the GS biotype (46% of absorbed) by 48 HAT. Although ^{14}C-glyphosate distribution patterns between biotypes at 24 HAT showed no major difference, the majority of translocated glyphosate accumulated in the SATL (which contains the apical meristem) in the GS biotype and in the SBTL in the resistant biotypes, C1B1 and T4B1, by 48 HAT (Table 5). Furthermore, more glyphosate (30% of absorbed) accumulated in the root of the T4B1 biotype 48 HAT, which was appreciably higher than that (13 and 15% of absorbed) in the roots of C1B1 and GS biotypes, respectively.

Efficacy of Single-Leaf–Treated Glyphosate on Whole Plant. Treating a single leaf with the 1× glyphosate rate, 0.84 kg ha^{-1}, as 10, 1-µl droplets resulted in 81% (of nontreated control plants) reduction in shoot fresh weight of the GS plants 3 WAT. Conversely, the C1B1 and T4B1 plants exhibited minimal injury symptoms with 0 and 18% reduction in shoot fresh weight, respectively (Table 6). Similar results were reported for horseweed (*Conyza canadensis* L. Cronq.) by Koger and Reddy (2005) and Italian ryegrass [*Lolium multiflorum* L. ssp. *multiflorum* (Lam.) Husnot] by Nandula et al. (2008). Susceptible horseweed biotypes were completely controlled (100%), glyphosate-resistant horseweed plants were controlled only 38 to 58% (Koger and Reddy 2005), and glyphosate-resistant Italian ryegrass plants were controlled only 35 to 55% (Nandula et al. 2008). The above results indicate differential movement of glyphosate between the resistant and susceptible biotypes.

Table 6. Efficacy of glyphosate on single-leaf–treated Palmer amaranth biotypes.[a]

Population	Shoot fresh weight reduction[b]
	%
GS[c]	81
C1B1	0
T4B1	18
LSD (0.05)	27

[a] Plants (10 cm tall, four to six leaves) were treated with 10 µl of a solution containing a commercial formulation of glyphosate (potassium salt) at a concentration of 0.84 kg ae ha^{-1} in 140 L of water. Ten 1-µl droplets of treatment solution were applied with a microsyringe on the adaxial surface of the third fully expanded leaf.
[b] Expressed as a percentage of aboveground fresh weight of nontreated plants.
[c] Abbreviation: GS, glyphosate-susceptible.

Shikimate Assay with Leaf Discs. Shikimate (shikimic acid) accumulation pattern in the Palmer amaranth biotypes is represented in Figure 3. A single regression model could not describe shikimate accumulation patterns in the three Palmer amaranth biotypes, C1B1, T4B1, and GS, in response to increasing glyphosate concentrations. A plausible reason could be the near-linear and low levels of shikimate in the C1B1 biotype and a nonlinear fashion of shikimate accumulation in the T4B1 and GS biotypes. It is apparent that the C1B1 biotype accumulated low levels of shikimate compared with the T4B1 and GS biotypes. Conversely, the pattern of shikimate buildup followed a similar trend in the T4B1 and GS biotypes. Different levels of accumulation of shikimate in the C1B1 and T4B1 biotypes suggest the likelihood of contrasting glyphosate resistance mechanisms. Culpepper et al. (2006) reported shikimate detection in a glyphosate-susceptible Palmer amaranth biotype after glyphosate treatment, but none in a resistant biotype. Steckel et al. (2008) documented shikimate accumulation in both glyphosate-resistant and -susceptible biotypes from Tennessee.

Glyphosate Metabolism. The levels of AMPA accumulated between the three Palmer amaranth biotypes when treated with glyphosate at 0.42 kg ha^{-1} were not significantly different, but there was a trend toward numerically higher AMPA levels in the C1B1 and T4B1 biotypes compared with the GS biotype

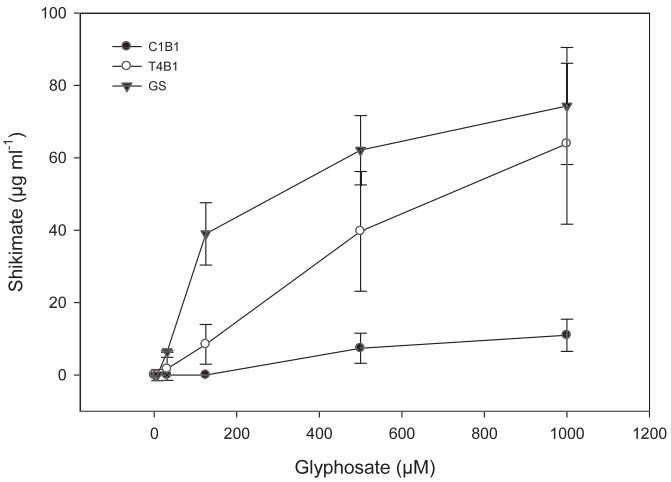

Figure 3. Effect of glyphosate concentration on shikimate levels in excised leaf discs of glyphosate-resistant C1B1 and T4B1 and glyphosate-susceptible Palmer amaranth biotypes. Error bars represent standard deviation of treatment means.

Table 7. Accumulation of aminomethylphosphonic acid (AMPA) in glyphosate-treated Palmer amaranth biotypes.[a]

Biotype	Glyphosate rate	AMPA	Glyphosate
	kg ae ha^{-1}	µg g^{-1} of tissue	
GS[b]	0.09	0.04	6.5
C1B1	1.52	0.40	75.3
T4B1	1.30	0.47	142.4
GS	0.42	0.05	28.7
C1B1	0.42	0.18	47.8
T4B1	0.42	0.36	55.8
LSD (0.05)[c]		NS	22

[a] Plants (10 cm tall, four to six leaves) were treated with a commercial formulation of glyphosate. Aboveground shoot biomass was harvested and analyzed 1 wk after treatment.
[b] Abbreviation: GS, glyphosate-susceptible.
[c] Only means of AMPA and glyphosate from plants treated with glyphosate at 0.42 kg ae ha^{-1} were compared.

(Table 7). The levels of glyphosate recovered were higher in the T4B1 plants compared with the GS biotype, with the glyphosate levels in the C1B1 biotype similar to those found in the other two biotypes. AMPA and glyphosate levels in Palmer amaranth plants treated with glyphosate at the respective GR_{50} rates of each biotype cannot be compared between biotypes because of different glyphosate rates used. A nearly fivefold decrease in glyphosate rate from 0.42 to 0.09 kg ha^{-1} did not seem to alter AMPA levels in the GS biotype. Similarly, a four- and threefold increase in glyphosate rate in C1B1 and T4B1, respectively, did not appreciably increase AMPA accumulation. These results indicate that glyphosate metabolism to AMPA had no role in defining resistance of the C1B1 and T4B1 biotypes to glyphosate. To date, glyphosate metabolism to AMPA imparting evolved resistance to glyphosate in a weed species has not been established (Duke 2011).

In summary, the above results confirm glyphosate resistance in Palmer amaranth from Mississippi. Also, two glyphosate-resistant biotypes exhibited low levels of multiple resistance to pyrithiobac, an ALS-inhibiting herbicide. Flumiclorac, a PPO inhibitor, was antagonistic to glyphosate in Palmer amaranth, and the antagonism was determined to be caused by reduced translocation of glyphosate by flumiclorac. Treating a single leaf with glyphosate in the form of tiny droplets resulted in significant reduction of growth in a susceptible biotype, but not in the resistant biotypes. This indirectly indicates differential glyphosate movement between the resistant and susceptible biotypes. ^{14}C-glyphosate studies indicated reduced absorption and translocation in the glyphosate-resistant biotypes. Metabolism of glyphosate to AMPA was low in both the resistant and susceptible biotypes, indicating an insignificant role in the resistance mechanism. One of the glyphosate-resistant biotypes, C1B1, accumulated negligible shikimate levels compared with another resistant biotype, T4B1, which recorded elevated shikimate levels comparable to the susceptible biotype. This indicates the possibility of different types of glyphosate resistance mechanisms in the resistant C1B1 and T4B1 biotypes at the target site level. Preliminary studies on the molecular mechanism of glyphosate resistance in C1B1 at the target site level indicated evidence of amplification of the *epsps* gene (Ribeiro et al. 2011). A glyphosate-resistant biotype from Georgia that had no difference in glyphosate absorption and translocation compared with a susceptible biotype (Culpepper et al. 2006) exhibited amplification of the *epsps* gene (Gaines et al. 2010).

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