

Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) confirmed in Georgia

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A glyphosate-resistant Palmer amaranth biotype was confirmed in central Georgia. In the field, glyphosate applied to 5- to 13-cm-tall Palmer amaranth at three times the normal use rate of 0.84 kg ae ha⁻¹ controlled this biotype only 17%. The biotype was controlled 82% by glyphosate at 12 times the normal use rate. In the greenhouse, *I*₅₀ values (rate necessary for 50% inhibition) for visual control and shoot fresh weight, expressed as percentage of the nontreated, were 8 and 6.2 times greater, respectively, with the resistant biotype compared with a known glyphosate-susceptible biotype. Glyphosate absorption and translocation and the number of chromosomes did not differ between biotypes. Shikimate was detected in leaf tissue of the susceptible biotype treated with glyphosate but not in the resistant biotype.

Nomenclature: Glyphosate; Palmer amaranth, *Amaranthus palmeri* S. Wats; AMA-PA.

Key words: Absorption, glyphosate resistance, herbicide resistance, resistance mechanism, translocation, weed resistance.

Palmer amaranth is among the three most troublesome weeds in Georgia cotton (*Gossypium hirsutum* L.), peanut (*Arachis hypogaea* L.), and soybean [*Glycine max* (L.) Merr.] and is among the top five most troublesome weeds in most other southeastern states (Webster 2005). It is an erect annual growing up to 2 m in height, and it produces unbranched terminal seedheads that can reach up to 0.5 m in length (Elmore 1990). Palmer amaranth is unique compared to many other *Amaranthus* species with its inflorescence being a terminal spike with male and female flowers on separate plants (dioecious) (Elmore 1990; Keeley et al. 1987). It is currently the most prevalent *Amaranthus* species in Georgia agronomic crops, which is likely in response to its competitiveness and aggressive growth habit and prolific seed production. Compared with common waterhemp (*Amaranthus rudis* S.), redroot pigweed (*A. retroflexus* L.), and tumble pigweed (*A. albus* L.), Palmer amaranth had the

greatest values for plant volume, dry weight, and leaf area (Horak and Loughin 2000). Additionally, rate of height increase per growing degree day for Palmer amaranth was 24 to 62% greater than for the other *Amaranthus* species.

A rapid growth rate and tall stature make Palmer amaranth extremely competitive with crops. Palmer amaranth reduced corn (*Zea mays* L.) yields 11 to 91% with 0.5 to 8 plants m⁻¹ of row (Massinga et al. 2001; Massinga and Currie 2002) and reduced soybean yield 17 to 68% with 0.33 to 10 plants m⁻¹ of row (Klingaman and Oliver 1994). Cotton lint yields in Texas decreased linearly from 13 to 54% as Palmer amaranth density increased from 1 to 10 plants in 9.1 m of row (Morgan et al. 2001). In Oklahoma (Rowland et al. 1999), cotton lint yield was reduced 5.9 to 11.5% for each Palmer amaranth plant in 10 m of row. Smith et al. (2000) reported that 3,260 Palmer amaranth plants ha⁻¹ reduced lint cotton yield and mechanical harvesting efficiency 22 and 2.4%, respectively.

Cotton is currently the dominant agronomic crop in Georgia (McKissick 2004). It is planted on 0.5 million ha, with greater than 94% of the acreage devoted to glyphosate-resistant cultivars (USDA-AMS 2004; USDA-ERS 2003). Traditional cotton herbicide programs that include cultivation, preplant incorporated and preemergence herbicides, plus postemergence-directed herbicides having both post-emergence and residual activity on Palmer amaranth, have been largely replaced by weed management systems often consisting only of glyphosate (Culpepper and York 1998; Nuti et al. 2003; Wilcut et al. 2003). Although control of emerged Palmer amaranth by glyphosate is excellent, continual emergence of the weed throughout the growing season, coupled with prolific seed production, enables it to replenish the seed bank and to spread rapidly (Jha and Norsworthy 2005; Keeley et al. 1987; Massinga et al. 2001). Multiple glyphosate applications are required for adequate season-long control of Palmer amaranth in glyphosate-resistant cotton (Everitt et al. 2003; Grichar et al. 2004; Keeling et al. 2004; Kendig and Nichols 2005; Nuti et al. 2003).

Monoculture production systems and repeated use of the same or similar herbicides have led to herbicide resistance in weeds (Peterson 1999; VanGessel 2001). Worldwide, there are currently 182 weed species with biotypes resistant to one or more herbicides (Heap 2005). In the late 1990s, some scientists thought weed resistance to glyphosate was unlikely because of unique properties of the herbicide, such as its mode of action, metabolism, chemical structure, and lack of residual activity in soil (Bradshaw et al. 1997). However, resistance to glyphosate has been confirmed in common ragweed (*Ambrosia artemisiifolia* L.), hairy fleabane [*Conyza bonariensis* (L.) Cronq.], horseweed [*C. canadensis* (L.) Cronq.], goosegrass [*Elusine indica* (L.) Gaertn.], Italian ryegrass (*Lolium multiflorum* Lam.), rigid ryegrass (*L. rigidum* Gaud.), and buckhorn plantain (*Plantago lanceolata* L.) (Heap 2005).

Since commercialization of glyphosate-resistant cotton in 1997, some Georgia growers have produced this cotton in a monoculture system and have relied exclusively on glyphosate applied multiple times each season to manage Palmer amaranth and other weeds. A cotton grower in Macon County, Georgia was unable to control Palmer amaranth with glyphosate in 2004. The objectives of our research were as follows: (1) to determine if the Macon County population of Palmer amaranth is resistant to glyphosate; (2) to quantify the level of glyphosate resistance; and (3) to understand the mechanism(s) allowing this biotype to tolerate glyphosate at rates known to be lethal to glyphosate-susceptible Palmer amaranth.

Materials and Methods

Field Experiment

The experiment was conducted in two fields 0.6 km apart near Oglethorpe, GA, in 2005. Both fields belonged to the aforementioned Macon County producer and fields had been treated with herbicides consisting of only pendimethalin, glyphosate, and paraquat during the previous 4 yr. Soils at both locations were 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. Cotton ('ST 5599BR')¹ was planted on May 10 in conventionally pre-

pared seedbeds. Plots consisted of four rows spaced 91 cm apart by 12 m. The experimental design was a randomized complete block with treatments replicated four times. Cultural practices, other than weed control, were according to local standards (Jost et al. 2005).

Treatments included the potassium salt of glyphosate² at 0, 1.25, 2.5, 5.0, 7.5, and 10.0 kg ae ha⁻¹ applied with a CO₂-pressurized backpack sprayer equipped with flat-fan nozzles delivering 140 L ha⁻¹ at 165 kPa and 4.8 km h⁻¹. Palmer amaranth heights and densities at time of treatment ranged from 5 to 10 cm and 30 plants m⁻², respectively, at one location and 7.5 to 13 cm and 120 plants m⁻² at the second location. Pitted morningglory (*Ipomoea lacunosa* L.) at less than 0.1 plant m⁻² was the only other weed species present, and it was controlled completely by all herbicide treatments. The entire trial area received S-metolachlor³ (1.0 kg ai ha⁻¹) 1 to 2 h after glyphosate application to prevent continual Palmer amaranth emergence. Visual control was estimated 28 d after application using a scale of 0 (no control) to 100 (plant death) (Frans et al. 1986).

Plant Materials for Greenhouse and Laboratory Experiments

Mature seeds from a single female Palmer amaranth plant surviving three glyphosate (0.84 kg ha⁻¹) applications were collected at one of the previously described Macon County, Georgia sites in the fall of 2004. The seeds (F1 generation) were hand cleaned and stored in a refrigerator at 1 C until use. Seeds from a known glyphosate-susceptible population of Palmer amaranth were collected from the University of Georgia Ponder Farm Research Station in Worth County and stored in a similar manner.

A preliminary glyphosate rate titration experiment was conducted twice in the greenhouse from January to March of 2005. Glyphosate at 0.3 kg ha⁻¹, applied to 5- to 7-cm plants, controlled the susceptible biotype of Palmer amaranth 97 to 100%. The suspected resistant biotype was controlled 23, 54, 75, 83, 93, and 100% by glyphosate at 0.6, 1.2, 2.4, 4.8, 7.2, and 9.6 kg ha⁻¹, respectively. Male and female plants from the suspected resistant biotype surviving glyphosate applied at 1.2 kg ha⁻¹ or greater were grown to maturity and crossed. Crossing was accomplished by shaking the inflorescence of male and female plants together every 2 d from pollen initiation until pollen was no longer visible. Mature seeds were harvested from female plants and stored at 1 C for at least 3 wk before planting. Seeds from the controlled crosses of resistant plants (F2 generation) and from the Ponder Farm were used for all greenhouse and laboratory experiments and are hereafter referred to as the resistant and susceptible biotypes, respectively.

Greenhouse Experiment

This experiment was conducted from July to September of 2005. The greenhouse was maintained at 32±5 C, and natural light was supplemented for 12 h each day by metal halide lamps (400 μE m⁻² s⁻¹). Seeds of the resistant and susceptible biotypes of Palmer amaranth were planted separately into round pots (15 cm in diameter, 15 cm deep) containing commercial potting media.⁴ Seedlings were thinned to one plant per pot within 2 d after emergence. Plants were watered by drip irrigation and were fertilized⁵ as needed to maintain good growth.

Seedlings 7 to 10 cm tall were treated with potassium salt of glyphosate at 0, 0.0375, 0.075, 0.15, 0.3, 0.6, 1.2, 2.4, 3.6, 4.8, 6.0, and 7.2 kg ha⁻¹. Glyphosate was applied with the backpack sprayer described for the field experiments. The experimental design was a randomized complete block (blocked by plant size) with treatments replicated five times, and the experiment was repeated once. Visible Palmer amaranth control was estimated 20 d after glyphosate application using a scale of 0 (no control) to 100 (complete control). At the final evaluation, plants were clipped at soil level and shoot fresh weights were determined.

Laboratory Experiments

¹⁴C-Glyphosate Absorption and Translocation

Glyphosate-resistant and -susceptible Palmer amaranth were grown in the greenhouse as described above. Plants were then moved into a growth chamber with a constant 28 C temperature and 50% relative humidity when they were 10 to 15 cm tall. Growth chamber lighting was provided by fluorescent and incandescent lamps at 450 μE m⁻² s⁻¹. Plants were allowed to acclimate for 2 d before treatment with glyphosate. The study was conducted as a randomized complete block design with treatments arranged as a split-plot and replicated five times. Whole plots were biotypes, and subplots were plant parts harvested. The study was repeated once.

The second fully expanded Palmer amaranth leaf (a source leaf) was covered with polyethylene film before treating the rest of the plant with potassium salt of glyphosate at 0.84 kg ha⁻¹ mixed with deionized water (Barnes and Oliver 2004; Bernards et al. 2005; Li et al. 2005; Lorraine-Colwill et al. 2002; Wakelin et al. 2004; Young et al. 2003). The film was then removed and the leaf was spotted with the radiolabeled solution using a microapplicator.⁶ The spotting solution was prepared by mixing 0.5 ml of the spray solution with ¹⁴C-labeled glyphosate (100:1, v/v). Technical grade phosphono-methyl-¹⁴C-glyphosate⁷ with 10,942 kBq mg⁻¹ specific activity and 99% radiochemical purity was used. Two 1-μl droplets of ¹⁴C-glyphosate were placed on the adaxial leaf surface approximately 2 mm away from the center vein, beginning at the base of the leaf and moving toward the center. Total specific activity applied contained approximately 2 kBq of radioactivity. Plants were returned to the growth chamber immediately after spotting. Plants were harvested 48 h after treatment. Research on common waterhemp indicated maximum glyphosate absorption 26 to 50 h after treatment (Li et al. 2005). Plants were cut at the soil line and sectioned into four parts: treated leaf, tissue above the treated leaf, tissue below the treated leaf, and roots. Soil was removed by washing the roots over a wire grid. Treated leaves were rinsed twice for 15 s with 5 ml of methanol/deionized water (1:1, v/v) to remove nonabsorbed ¹⁴C-glyphosate (Li et al. 2005). A 1-ml aliquot of the combined rinsates was added to 10 ml of scintillation fluid,⁸ and radioactivity was quantified by liquid scintillation spectrometry.⁹ All plant parts were dried for 48 h at 45 C, weighed, and combusted with a biological sample oxidizer.¹⁰ Radioactivity in the oxidized samples was quantified by liquid scintillation spectrometry. The amount of herbicide absorbed was calculated as the total radioactivity recovered from oxidation of the four plant parts and expressed as a percentage of the total radioactivity applied. Distribution of

¹⁴C-glyphosate in various plant parts was expressed as the percentage of total absorbed radioactivity.

In Vivo Shikimate Assay

Glyphosate-resistant and -susceptible plants were grown in the greenhouse as previously described. Shikimate was determined according to a modification of the method of Gaitonde and Gordon (1958), Koger et al. (2005), and Shaner et al. (2005). Six leaf discs (3 mm in diameter) per plant from the youngest fully formed leaf of each biotype were excised and placed in a 1-ml solution containing 8.4, 42, or 84.5 mg L⁻¹ of potassium salt of glyphosate for 16 h at 25 C under supplemental light (400 μE m⁻² s⁻¹). Leaf discs were then placed in 0.4 ml of 0.25 N HCl for 60 min after which a 100-μl aliquot was mixed with 0.4 ml 0.25% periodic acid with 0.25% metaperiodate solution for 60 min. After the periodic acid–metaperiodate incubation, a 0.4-ml aliquot of 0.6 M sodium hydroxide with 0.22 M sodium sulfite solution was added. Optical density of the solution at 380 nm was determined spectrophotometrically.¹¹ A shikimate standard curve was developed by adding known amounts of shikimate¹² to vials containing leaf discs not exposed to glyphosate. Shikimate levels are reported as micrograms of shikimate per milliliter of HCl solution. Treatments were replicated three times, and the study was repeated three times.

Ploidy Determination

Nuclear DNA content of developing, non-fully expanded leaves of greenhouse-grown glyphosate-resistant and -susceptible Palmer amaranth was measured by flow cytometry. Samples were prepared following the methods outlined by Morgan et al. (1998). Leaf tissue from four glyphosate-resistant and -susceptible plants was chopped at room temperature using a razor blade in 0.5 ml of isolation medium (high-resolution DNA kit solution A, type T: DNA isolation).¹³ The suspension was filtered through a 40-μm mesh nylon filter and mixed with four- to fivefold volume of staining solution (high-resolution DNA kit solution B, type T: staining) with DAPI (4',6-diamidino-2-phenylindole) as the DNA-specific fluorochrome. The nuclear suspension was analyzed on a PAS-III flow cytometer¹³ with 100-W high pressure mercury lamp; KG1, BG38, UG1, OG515 filters; TK 560 mirror; and GG 435 as barrier filter. Eleven thousand nuclei per plant sample were analyzed.

Statistical Analysis

Data from field and greenhouse experiments, which utilized a series of glyphosate rates, were subjected to nonlinear regression in addition to ANOVA. Visible Palmer amaranth control and shoot fresh weight, expressed as a percentage of the nontreated control, were regressed against the log₁₀ of the glyphosate rate (SAS 1999). The intent was to determine if the response could be described by the log-logistic dose–response curve (equation [1]), where C = lower limit, D = upper limit, b = slope, and I_{50} = dose giving 50% response (Seefeldt et al. 1995).

$$y = C + \frac{D - C}{1 + (x/I_{50})^b} \quad [1]$$

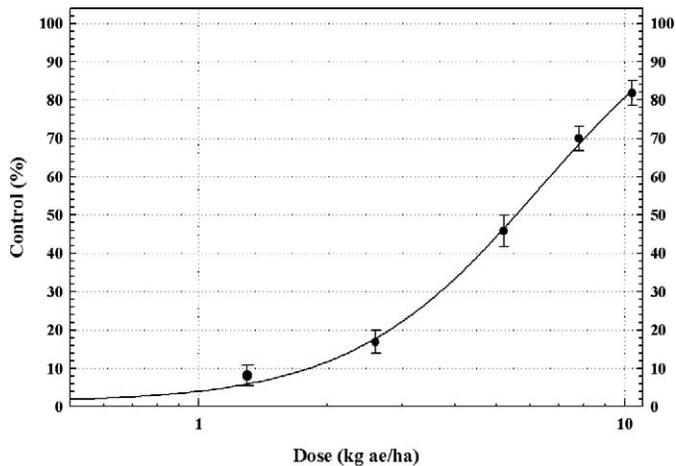


FIGURE 1. Glyphosate-resistant Palmer amaranth control 28 d after glyphosate application in the field. Log-logistic dose-response curve:

$$y = 112 + \frac{-0.36 - 112}{1 + (x/6.1)^{2.0}} \quad R^2 = 0.94,$$

$$I_{50} = 6.1 \text{ kg ha}^{-1}$$

The log-logistic curve is often used in dose-response studies where the dose (i.e., rate) ranges from no effect to complete death (Gad and Weil 1989; Seefeldt et al. 1995). Constants generated by statistical software (SAS 1999) allowed the equation to be solved, and the glyphosate rates required to produce 50% visual control and 50% fresh weight reduction (I_{50}) were determined. For presentation,¹⁴ parameters were fitted with a sigmoidal response curve which had been previously generated.

All other data were subjected to ANOVA using the general linear models of SAS (1999). Within each experiment, data were combined for analysis because there were no treatment by study repetition interactions. In laboratory experiments, means were separated by Fisher's Protected LSD test at the 0.05 probability level and the standard error of the mean was calculated. For the shikimate assay, standard error of the means and a linear regression of the resulting shikimate values versus glyphosate concentration were computed for the susceptible biotype. Shikimate was not detectable in the glyphosate-resistant biotype, hence standard error and R^2 values are not reported for this biotype.

Results and Discussion

Field Experiment

Control of the suspected resistant Palmer amaranth biotype by glyphosate was described with the log-logistic dose-response curve (Figure 1). Glyphosate at 1.25, 2.5, 5.0, 7.5, and 10.0 kg ha⁻¹ controlled emerged Palmer amaranth 8, 17, 46, 70, and 82%, respectively, 28 d after application. Glyphosate is recommended at 0.84 kg ha⁻¹ for control of *Amaranthus* species up to 46 cm in height (Anonymous 2005; Jost et al. 2005), a rate which normally controls *Amaranthus* species well (Everitt et al. 2003; Grichar et al. 2004; Keeling et al. 2004; Kendig and Nichols 2005; Nuti et al. 2003). In our experiment, glyphosate at 12 times the recommended rate, or 10 kg ha⁻¹, failed to provide commercially acceptable control.

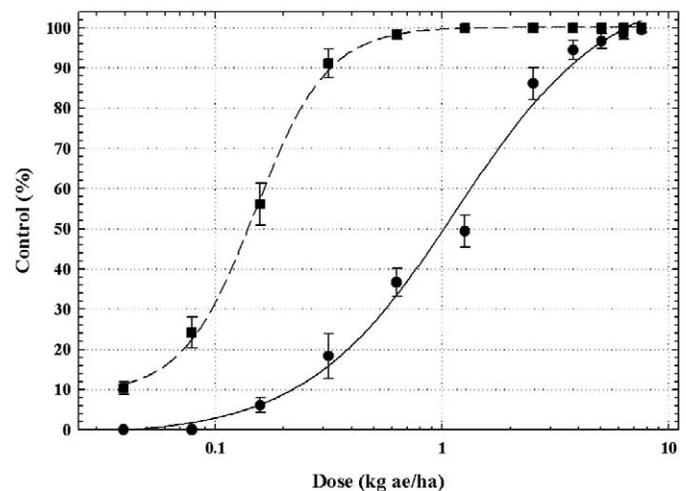


FIGURE 2. Visual control of glyphosate-resistant and -susceptible Palmer amaranth 20 d after glyphosate application in the greenhouse. Log-logistic dose-response curves:

Resistant

$$y = 111 + \frac{-1.15 - 111}{1 + (x/1.2)^{1.3}} \quad R^2 = 0.95,$$

$$I_{50} = 1.2 \text{ kg ha}^{-1}$$

Susceptible

$$y = 100 + \frac{9.0 - 100}{1 + (x/0.15)^{2.7}} \quad R^2 = 0.96,$$

$$I_{50} = 0.15 \text{ kg ha}^{-1}$$

Poor control of Palmer amaranth in this experiment was not due to environmental stress on the plants at time of application. During the week prior to treatment, four rainfall events totaling 6.5 cm occurred. Poor control also was not due to Palmer amaranth emerging after glyphosate application. Rainfall occurred 1, 11, 19, 21, and 26 d after herbicide application, with 4.5, 3.7, 1.4, 1.8, and 1.1 cm of rainfall, respectively. These conditions contributed to excellent residual control by *S*-metolachlor. No new emergence of Palmer amaranth was noted during the 28-d evaluation period following glyphosate application.

Greenhouse Experiment

This experiment, in conjunction with the field experiment, confirmed that the Palmer amaranth infesting the farm in Macon County, Georgia is indeed resistant to glyphosate. Log-logistic dose-response curves described visual control of both glyphosate-resistant and -susceptible biotypes (Figure 2). The I_{50} parameter estimate for visual control of the susceptible biotype was 0.15 kg ha⁻¹, while the I_{50} for visual control of the resistant biotype was eightfold greater, or 1.2 kg ha⁻¹. Complete control of the susceptible biotype was noted with glyphosate at 0.6 kg ha⁻¹ while a 12-fold increase in glyphosate rate (7.2 kg ha⁻¹) was necessary for complete control of the resistant biotype. Glyphosate-resistant Palmer amaranth was not affected by glyphosate at rates below 0.15 kg ha⁻¹; however, as rates increased above 0.15 kg ha⁻¹, the degree of plant stunting and shoot apex chlorosis increased. Most resistant plants resumed growth within 7 d after glyphosate application at rates less than 2.4 kg ha⁻¹. Continued growth occurred pri-

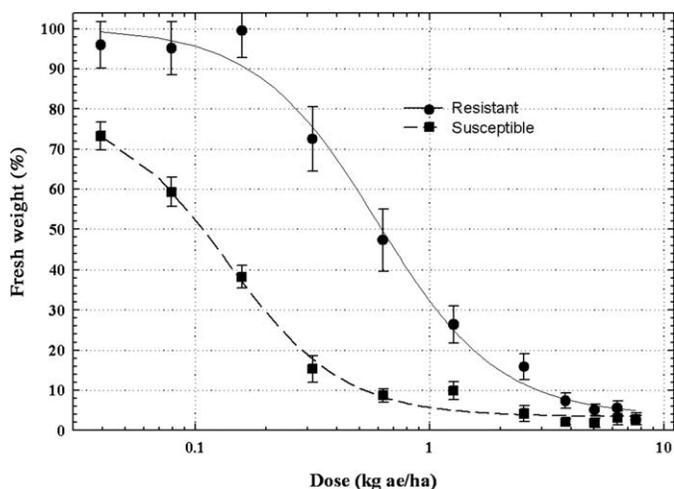


FIGURE 3. Glyphosate-resistant and -susceptible Palmer amaranth shoot fresh weights, as a percentage of the nontreated check, 20 d after glyphosate application in the greenhouse. Log-logistic dose-response curves:

Resistant

$$y = 114 + \frac{-0.12 - 114}{1 + (x/0.56)^{1.7}} \quad R^2 = 0.87,$$

$$I_{50} = 0.56 \text{ kg ha}^{-1}$$

Susceptible

$$y = 91 + \frac{0.93 - 91}{1 + (x/0.09)^{2.8}} \quad R^2 = 0.88,$$

$$I_{50} = 0.09 \text{ kg ha}^{-1}$$

marily from the apical growing point but did occasionally occur in axillary growing points in plants surviving high glyphosate rates (4.8 and 6.0 kg ha⁻¹).

Glyphosate-resistant and -susceptible Palmer amaranth shoot fresh weights, expressed as a percentage of nontreated plants, were also described with the log-logistic dose-response curve (Figure 3). Absolute values for the *I*₅₀ parameter estimates for shoot fresh weights were approximately half the *I*₅₀ values for visual control, but trends for fresh weight and visual control were similar. The *I*₅₀ value for shoot fresh weight of glyphosate-susceptible Palmer amaranth was 0.09 kg ha⁻¹ while the *I*₅₀ for the resistant biotype was 6.2-fold greater, or 0.56 kg ha⁻¹. Greater *I*₅₀ values for visual control compared with shoot fresh weight are likely due to impacts of glyphosate on plant development (cellular development such as leaf and stem thickness as well as the plants' ability to maintain normal hydration levels) that could not be visually detected. No difference in shoot fresh weight between biotypes was noted in the absence of glyphosate (data not shown).

Laboratory Experiments

Absorption and Translocation

Approximately 90% of the total applied radioactivity was recovered from leaf washes and oxidation of plant parts. No differences in ¹⁴C absorption were noted 48 h after application to either biotype (Table 1). Glyphosate-resistant and -susceptible plants absorbed 36.4 and 31.2% of the applied herbicide, respectively. Li et al. (2005) reported 40 to 65% ¹⁴C-glyphosate absorption by common waterhemp 26 to 50 h after application.

TABLE 1. Absorption and distribution of ¹⁴C 48 h after ¹⁴C-glyphosate application to glyphosate-resistant and -susceptible Palmer amaranth.

Biotype	Absorption ^b	Distribution ^a			
		Treated leaf	Above treated leaf	Below treated leaf	Roots
		%			
Resistant	36.4 a ^c	58.2 a	12.9 a	16.2 a	12.6 a
Susceptible	31.2 a	66.2 a	9.6 a	16.1 a	8.0 a

^a Distribution expressed as percentage of absorbed ¹⁴C.

^b Absorption expressed as percentage of total ¹⁴C applied.

^c Values within a column followed by the same letter do not differ significantly according to Fisher's Protected LSD 0.05.

Translocation of ¹⁴C-glyphosate out of the treated leaf and distribution throughout the plant did not differ between glyphosate-resistant and -susceptible Palmer amaranth biotypes (Table 1). Resistant and susceptible plants translocated 41.7 and 33.7%, respectively, of the applied ¹⁴C-glyphosate out of the treated leaf.

Ploidy Determination

Previous studies demonstrated significant variability in genome size across *Amaranthus* species, with Palmer amaranth possessing the smallest genome of six tested *Amaranthus* species (Jeschke et al. 2003; Rayburn et al. 2005). Glyphosate-resistant and -susceptible Palmer amaranth had similar genome sizes, as indicated by overlapping peaks in the histogram for the amount of DNA in the nuclei (data not shown). Bunnell et al. (2003) indicated that higher numbers of chromosomes were suspected to increase herbicide tolerance in bahiagrass (*Paspalum notatum*). Tetraploid bahiagrass is tolerant to metsulfuron, while diploid bahiagrass is susceptible to this herbicide. Our results suggest no difference in ploidy level between glyphosate-resistant and -susceptible Palmer amaranth biotypes.

In Vivo Shikimate Assay

Glyphosate competes with the substrate phosphoenolpyruvate for a binding site on the enzyme 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS, E.C.2.5.1.19), resulting in uncontrolled flow of carbon and subsequent accumulation of shikimate in affected sensitive tissues (Amrhein et al. 1980). Accumulation of shikimate in glyphosate-treated plants indicates the herbicide is affecting the activity of EPSPS (Mueller et al. 2003). In our experiment, shikimate was detected in leaf tissue of glyphosate-susceptible Palmer amaranth at the lowest concentration of glyphosate examined (8.4 mg L⁻¹), and shikimate concentration increased linearly as glyphosate concentration increased (Figure 4). Shikimate was not detected in leaf tissue of glyphosate-resistant Palmer amaranth regardless of the glyphosate concentration.

Our results suggest that the glyphosate-resistant Palmer amaranth biotype from central Georgia possesses a different mechanism of resistance than glyphosate-resistant horseweed and rigid ryegrass biotypes that have thus far been described. No differences were noted in ¹⁴C-glyphosate absorption between resistant and susceptible biotypes of horseweed (Feng

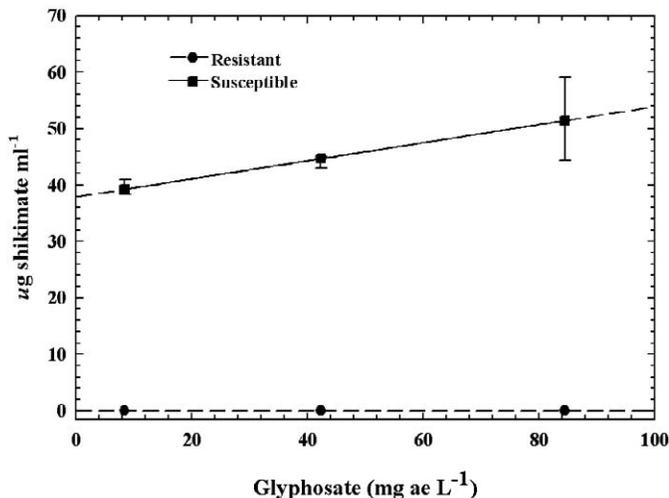


FIGURE 4. Effect of glyphosate concentration on shikimate levels in leaf discs from glyphosate-resistant and -susceptible Palmer amaranth biotypes. Bars indicate standard error of the mean.

et al. 2004; Koger and Reddy 2005) and rigid ryegrass (Wakelin et al. 2004). Limited translocation of glyphosate out of treated leaves, however, was observed with resistant biotypes of both species. Shikimate accumulated in treated leaves of glyphosate-resistant horseweed, indicating resistance was not due to an altered target site (Koger et al. 2005; Mueller et al. 2003). Differential glyphosate absorption or translocation by the glyphosate-resistant biotype of Palmer amaranth does not appear to explain the observed resistance. Rather, the lack of shikimate accumulation in glyphosate-treated leaves indicates the mechanism of resistance is an altered target site. Further research is needed to confirm this.

The level of resistance to glyphosate in the Georgia Palmer amaranth biotype (six- to eightfold in whole plants) is less than that often observed in biotypes resistant to other modes of herbicide action (Ferguson et al. 2001; Mallory-Smith et al. 1990; Smisek et al. 1998). It is, however, similar to that in other species confirmed to be resistant to glyphosate (Mueller et al. 2003; VanGessel 2001). Regardless of the level of resistance, a grower's ability to manage this biotype of Palmer amaranth with glyphosate no longer exists.

This is the world's first confirmed case of glyphosate resistance in an *Amaranthus* species (Heap 2005; HRAC 2005), and it is a significant finding with serious ramifications for future weed management. Palmer amaranth is already one of the most troublesome weeds of agronomic crops across the southern United States (Webster 2005); resistance to glyphosate will only exacerbate the problem, especially in light of the widespread planting of glyphosate-resistant crops. Rapid spread by pollen is expected in this dioecious species (forced outcrossing). Moreover, resistance to other herbicides, such as dinitroanilines and acetolactate synthase inhibitors (Heap 2005), limits the options to control glyphosate-resistant Palmer amaranth.

Other *Amaranthus* species are prevalent in all regions of North America, especially in the midwestern and southern areas of the United States where glyphosate-resistant crops have been broadly adopted. *Amaranthus* species, including Palmer amaranth, can outcross with other related monoecious and dioecious species (Franssen et al. 2001; Tranel et

al. 2002; Trucco et al. 2005). Glyphosate resistance in this particular Palmer amaranth population will likely spread to other adjacent *Amaranthus* species by outcrossing, limited only by movement of viable pollen in the atmosphere.

Sources of Materials

¹ ST 5599BR cotton, Monsanto Co., 800 North Lindbergh Avenue, St. Louis, MO 63167.

² Roundup WEATHERMAX™, potassium salt of glyphosate, Monsanto Co., 800 North Lindbergh Avenue, St. Louis, MO 63167.

³ Dual Magnum™, Syngenta Crop Protection, Inc., 410 South Swing Road, Greensboro, NC 27419.

⁴ Metro Mix 200 growing medium, Scotts-Sierra Horticultural Products Co., 14111 Scottslawn Road, Marysville, OH 43041.

⁵ Peters Professional All Purpose 20–20–20 fertilizer, Scotts-Sierra Horticultural Products Co., 14111 Scottslawn Road, Marysville, OH 43041.

⁶ Burkard Manufacturing Co. Ltd., Woodcock Hill Industrial Estate, Rickmansworth, Hertfordshire WD3 1PJ, U.K.

⁷ Technical grade ¹⁴C-glyphosate, American Radiolabeled Chemicals, Inc., 11624 Bowling Green Drive, St. Louis, MO 63146.

⁸ ScintiSafe 30% scintillation cocktail, Fisher Chemicals, 1 Reagent Lane, Fairlawn, NJ 07410.

⁹ Model LS 6000 TA liquid scintillation spectrophotometer, Beckman Instruments, Inc., 2500 Harbor Boulevard, Fullerton, CA 92634-3100.

¹⁰ Model OX-500 biological material oxidizer, R. J. Harvey Instrument Corp., 123 Patterson Street, Hillsdale, NJ 07642.

¹¹ Shimadzu UV-1601 UV/Vis spectrophotometer, Shimadzu Instrument, 7102 Riverwood Drive, Columbia, MD 21046.

¹² Shikimic acid, Sigma, P.O. Box 14508, St. Louis, MO 63178.

¹³ Flow cytometer and isolation medium, Partec GmbH, Otto-Hahn-Str. 32, D-48161 Münster, Germany.

¹⁴ SigmaPlot 4® for Windows®, SPSS Inc., 444 N Michigan Ave., Chicago, IL 60611.

Literature Cited

- Amrhein, N., B. Deus, P. Gehrke, and H. C. Steinrucken. 1980. The site of inhibition of the shikimate pathway by glyphosate. II. Interference of glyphosate with chorismate formation *in vivo* and *in vitro*. *Plant Physiol.* 66:830–834.
- Anonymous. 2005. Roundup WEATHERMAX™ herbicide specimen label. www.cdms.net/ldat/ld5UJ024.pdf.
- Barnes, J. W. and L. R. Oliver. 2004. Cloransulam absorption, translocation, and efficacy on common broadleaf weed species. *Weed Sci.* 52: 634–641.
- Bernards, M. L., K. D. Thelen, D. Penner, R. B. Muthukumar, and J. L. McCracken. 2005. Glyphosate interaction with manganese in tank mixtures and its effect on glyphosate absorption and translocation. *Weed Sci.* 53:787–794.
- Bradshaw, L. D., S. R. Padgett, S. L. Kimball, and B. H. Wells. 1997. Perspectives on glyphosate resistance. *Weed Technol.* 11:189–198.
- Bunnell, B. T., R. D. Baker, L. B. McCarty, D. W. Hall, and D. L. Colvin. 2003. Differential responses of five bahiagrass (*Paspalum notatum*) cultivars to metsulfuron. *Weed Technol.* 17:550–553.
- Culpepper, A. S. and A. C. York. 1998. Weed management in glyphosate-tolerant cotton. *J. Cotton Sci.* 2:174–185.
- Elmore, C. D. 1990. *Weed Identification Guide*. Champaign, IL: Southern Weed Science Society.
- Everitt, J. D., J. W. Keeling, L. L. Lyon, and P. A. Dotray. 2003. Palmer amaranth (*Amaranthus palmeri*) and devil's-claw (*Proboscidea louisianica*) control with Staple/glyphosate combinations in Roundup Ready cotton. Page 2262 in *Proceedings of the Beltwide Cotton Conference*. Memphis, TN: National Cotton Council of America.
- Feng, P.C.C., M. Tran, T. Chiu, R. D. Sammons, G. R. Heck, and C. A. CaJacob. 2004. Investigation into glyphosate-resistant horseweed (*Co-*

- nyza canadensis*): retention, uptake, translocation, and metabolism. *Weed Sci.* 52:498–505.
- Ferguson, G. M., A. S. Hamill, and F. J. Tardif. 2001. ALS inhibitor resistance in populations of Powell amaranth and redroot pigweed. *Weed Sci.* 49:448–453.
- Frans, R. E., R. Talbert, D. Marx, and H. Crowley. 1986. Experimental design and techniques for measuring and analyzing plant responses to weed control practices. Pages 29–46 in N. D. Camper, ed. *Research Methods in Weed Science*. Champaign, IL: Southern Weed Science Society.
- Franssen, A. S., D. Z. Skinner, K. Al-Khatib, M. J. Horak, and P. A. Kulakow. 2001. Interspecific hybridization and gene flow of ALS resistance in *Amaranthus* species. *Weed Sci.* 49:598–606.
- Gad, S. C. and C. S. Weil. 1989. *Statistics for toxicologists*. Pages 435–484 in A. W. Hayes, ed. *Principles and Methods of Toxicology*. New York: Raven.
- Gaitonde, M. and M. Gordon. 1958. A microchemical method for detecting and determination of shikimic acid. *J. Biol. Chem.* 230:1043–1050.
- Grichar, W. J., B. A. Besler, K. D. Brewer, and B. W. Minton. 2004. Using soil-applied herbicides in combination with glyphosate in a glyphosate-resistant cotton herbicide program. *Crop Protect.* 23(10):1007–1010.
- Heap, I. M. 2005. International Survey of Herbicide Resistant Weeds. www.weedresearch.org.
- Horak, M. J. and T. M. Loughin. 2000. Growth analysis of four *Amaranthus* species. *Weed Sci.* 48:347–355.
- [HRAC] Herbicide Resistant Action Committee. 2005. Guideline to the Management of Herbicide Resistance. www.plantprotection.org/hrac/guideline.html.
- Jeschke, M. R., P. J. Tranel, and A. L. Rayburn. 2003. DNA content analysis of smooth pigweed (*Amaranthus hybridus*) and tall waterhemp (*A. tuberculatus*): implications of hybrid detection. *Weed Sci.* 51:1–3.
- Jha, P. and J. K. Norsworthy. 2005. Effect of soybean canopy formation and tillage on temporal emergence of Palmer amaranth. *Proc. South. Weed Sci. Soc.* 58:214.
- Jost, P. J., S. M. Brown, S. Culpepper, G. Harris, B. Kemerait, P. Roberts, D. Shurley, and J. Williams. 2005. 2005 Georgia Cotton Production Guide. Tifton, GA: University of Georgia Cooperative Extension Service Publ. CSS-05-01. 100 p.
- Keeley, P. E., C. H. Carter, and R. J. Thullen. 1987. Influence of planting date on growth of Palmer amaranth (*Amaranthus palmeri*). *Weed Sci.* 35:199–204.
- Keeling, J. W., J. D. Everitt, and P. A. Dotray. 2004. Application timings and rates in Roundup Ready Flex cotton. *Proc. South. Weed Sci. Soc.* 57:254.
- Kendig, J. A. and R. L. Nichols. 2005. Palmer amaranth (*Amaranthus palmeri*) control. Page 2897 in *Proceedings of the Beltwide Cotton Conference*. Memphis, TN: National Cotton Council of America.
- Klingaman, T. E. and L. R. Oliver. 1994. Palmer amaranth (*Amaranthus palmeri*) interference in soybeans (*Glycine max*). *Weed Sci.* 42:523–527.
- Koger, C. H. and K. N. Reddy. 2005. Role of absorption and translocation in the mechanism of glyphosate resistance in horseweed (*Conyza canadensis*). *Weed Sci.* 53:84–89.
- Koger, C. H., D. L. Shaner, W. B. Henry, T. Nadler-Hassar, W. E. Thomas, and J. W. Wilcut. 2005. Assessment of two nondestructive assays for detecting glyphosate resistance in horseweed (*Conyza canadensis*). *Weed Sci.* 53:559–566.
- Li, J., R. J. Smeda, B. A. Sellers, and W. G. Johnson. 2005. Influence of formulation and glyphosate salt on absorption and translocation in three annual weeds. *Weed Sci.* 53:153–159.
- Lorraine-Colwill, D. E., S. B. Powles, T. R. Hawkes, P. H. Hollinshead, S.A.J. Warner, and C. Preston. 2002. Investigations into the mechanism of glyphosate resistance in *Lolium rigidum*. *Pest. Biochem. Physiol.* 74:62–72.
- Mallory-Smith, C. A., D. C. Thill, and M. J. Dial. 1990. Identification of sulfonyleurea herbicide-resistant prickly lettuce (*Lactuca serriola*). *Weed Technol.* 4:163–168.
- Massinga, R. A. and R. S. Currie. 2002. Impact of Palmer amaranth (*Amaranthus palmeri*) on corn (*Zea mays*) grain yield and yield and quality of forage. *Weed Technol.* 16:532–536.
- Massinga, R. A., R. S. Currie, M. J. Horak, and J. Boyer Jr. 2001. Interference of Palmer amaranth in corn. *Weed Sci.* 49:202–208.
- McKissick, J. 2004. 2003 Georgia Farm Gate Value Report. Athens, GA: The University of Georgia College of Agricultural and Environmental Sciences Area Report No. 04-01. 181 p.
- Morgan, G. D., P. A. Baumann, and J. M. Chandler. 2001. Competitive impact of Palmer amaranth (*Amaranthus palmeri*) on cotton (*Gossypium hirsutum*) development and yield. *Weed Technol.* 15:408–412.
- Morgan, R. N., P. Ozias-Akins, and W. W. Hanna. 1998. Seed set in an apomictic BC₃ pearl millet. *Int. J. Plant Sci.* 159:89–97.
- Mueller, T. C., J. H. Massey, R. M. Hayes, C. L. Main, and C. N. Stewart Jr. 2003. Shikimate accumulation in both glyphosate-sensitive and glyphosate-resistant horseweed (*Conyza canadensis*). *J. Agric. Food Chem.* 51:680–684.
- Nuti, R., A. York, J. Bachele, and K. Edmisten. 2003. Pest control costs and returns in conventional and transgenic cotton management systems. Page 2262 in *Proceedings of the Beltwide Cotton Conference*. Memphis, TN: National Cotton Council of America.
- Peterson, D. E. 1999. The impact of herbicide-resistant weeds on Kansas agriculture. *Weed Technol.* 13:632–635.
- Rayburn, A. L., R. McCloskey, T. C. Tatum, G. A. Bollero, M. R. Jeschke, and P. J. Tranel. 2005. Genome size analysis of weedy *Amaranthus* species. *Crop Sci.* 45:2557–2562.
- Rowland, M. W., D. S. Murry, and L. M. Verhalen. 1999. Full-season Palmer amaranth (*Amaranthus palmeri*) interference with cotton (*Gossypium hirsutum*). *Weed Sci.* 47:305–309.
- [SAS] Statistical Analysis Systems. 1999. *SAS/STAT User's Guide*, Version 8. Cary, NC: Statistical Analysis System Institute Inc. 3884 p.
- Seefeldt, S. S., J. E. Jensen, and E. P. Fuerst. 1995. Log-logistic analysis of herbicide dose-response relationships. *Weed Technol.* 9:218–227.
- Shaner, D. L., T. Nadler-Hassar, W. B. Henry, and C. H. Koger. 2005. A rapid *in vivo* shikimate accumulation assay with excised leaf discs. *Weed Sci.* 53:769–774.
- Smisek, A., C. Doucet, M. Jones, and S. Weaver. 1998. Paraquat resistance in horseweed (*Conyza canadensis*) and Virginia pepperweed (*Lepidium virginicum*) from Essex County, Ontario. *Weed Sci.* 46:200–204.
- Smith, D. T., R. V. Baker, and G. L. Steele. 2000. Palmer amaranth (*Amaranthus palmeri*) impacts on yield, harvesting, and ginning in dryland cotton (*Gossypium hirsutum*). *Weed Technol.* 14:122–126.
- Tranel, P. J., J. J. Wassom, M. R. Jeschke, and A. L. Rayburn. 2002. Transmission of herbicide resistance from a monococious to a dioecious weedy *Amaranthus* species. *Theor. Appl. Genet.* 105:674–679.
- Trucco, F., M. R. Jeschke, A. L. Rayburn, and P. J. Tranel. 2005. Promiscuity in weedy amaranths: high frequency of female tall waterhemp (*Amaranthus tuberculatus*) × smooth pigweed (*A. hybridus*) hybridization under field conditions. *Weed Sci.* 53:46–54.
- [USDA-AMS] United States Department of Agriculture—Agricultural Marketing Service—Cotton Program. 2004. *Cotton Varieties Planted 2004* Crop. Memphis, TN.
- [USDA-ERS] United States Department of Agriculture—Economic Research Service. 2003. *The Extent of Adoption of Bioengineered Crops*. www.ers.usda.gov/publications/aer810d.pdf.
- VanGessel, M. J. 2001. Glyphosate-resistant horseweed from Delaware. *Weed Sci.* 49:703–705.
- Wakelin, A. M., D. F. Lorraine-Colwill, and C. Preston. 2004. Glyphosate resistance in four different populations of *Lolium rigidum* is associated with reduced translocation of glyphosate to meristematic zones. *Weed Res.* 44:453–459.
- Webster, T. M. 2005. Weed survey—southern states: broadleaf crops subsection. *Proc. South. Weed Sci. Soc.* 58:291–304.
- Wilcut, J. W., R. L. Hayes, R. L. Nichols, et al. 2003. A beltwide regional economic assessment of weed management systems in non-transgenic and transgenic cotton. Page 2260 in *Proceedings of the Beltwide Cotton Conference*. Memphis, TN: National Cotton Council of America.
- Young, B. G., A. W. Knepp, L. M. Wax, and S. E. Hart. 2003. Glyphosate translocation in common lambsquarters (*Chenopodium album*) and velvetleaf (*Abutilon theophrasti*) in response to ammonium sulfate. *Weed Sci.* 51:151–156.

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