Weed Science, 53:84–89. 2005

Role of absorption and translocation in the mechanism of glyphosate resistance in horseweed (*Conyza canadensis*)

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Greenhouse and laboratory experiments were conducted to investigate mechanisms of glyphosate resistance in horseweed populations from Mississippi, Arkansas, Delaware, and Tennessee. A nondestructive leaf-dip bioassay was developed to confirm resistance and susceptibility in individual test plants. A single leaf was excised from each plant, and the petiole and bottom one-fourth of leaf was dipped in a 600 mg ae L^{-1} glyphosate solution for 2 d followed by visually estimating the injury on a scale of 0 to 10. Plants were classified as resistant (R) if the score was 2 to 3 and susceptible (S) if the score was 5 to 6. ^{14}C-glyphosate solution was applied on the adaxial surface of a fully expanded leaf of the second whorl of four-whorl rosette plants. Plants were harvested 48 h after treatment and radioactivity was determined in treated leaf, other leaves, crown, and roots. Absorption of ^{14}C-glyphosate was similar (47 to 54%) between R and S plants from within and among the four states, suggesting absorption is not involved in glyphosate resistance. The amount of radioactivity translocated from the treated leaf was reduced in R plants compared with S plants. The reduction in translocation of ^{14}C-glyphosate ranged from 28% in Mississippi-R biotype to 48% in Delaware-R biotype compared with their respective S biotypes. Epicuticular wax mass ranged from 6 to 80 μg cm^{-2} among horseweed biotypes, with no differences between R and S biotypes within each state. Treating two leaves with glyphosate solution at the field use rate (0.84 kg ae ha^{-1}) killed S plants but not R plants (38 to 58% control) regardless of state origin. These results suggest that a simple bioassay can be used to screen biotypes for suspected resistance and that reduced translocation of glyphosate plays a major role in glyphosate resistance in R biotypes of horseweed.

Nomenclature: ^{14}C-glyphosate; glyphosate; horseweed, *Conyza canadensis* (L.) Cronq.

Key words: Bioassay, cuticle, epicuticular wax, glyphosate, glyphosate resistance, herbicide efficacy, weed resistance.

Glyphosate resistance in horseweed was first reported in Delaware in 2001 (VanGessel 2001). Since 2001, glyphosate-resistant horseweed biotypes have been reported in Tennessee (Mueller et al. 2003), Mississippi (Koger et al. 2004), and Kentucky, Indiana, Maryland, New Jersey, Ohio, Arkansas, and North Carolina (Heap 2004). Aside from horseweed, five other weed species have developed resistance to glyphosate in recent years. Evolved glyphosate resistance in goosegrass [*Eleusine indica* (L.) Gaertn.] from Malaysia; Italian ryegrass (*Lolium multiflorum* Lam.) from Brazil and Chile; rigid ryegrass (*Lolium rigidum* Gaud.) from Australia, South Africa, and United States; and buckhorn plantain (*Plantago lanceolata* L.) and hairy fleabane [*Conyza bonariensis* (L.) Crong.] from South Africa have been documented (Heap 2004; Lee and Ngim 2000; Powles et al. 1998; Pratley et al. 1999). Repeated use of glyphosate over years and increased exposure of weed populations to frequent applications of glyphosate in glyphosate-resistant crops may have contributed to high selection pressure and subsequent development of weeds resistant to glyphosate.

The mechanism of herbicide resistance in weeds is generally due to reduced herbicide absorption, reduced translocation of herbicide from the site of absorption to the target site, rapid metabolic detoxification of herbicide, and altered herbicide target site. Weed species can be resistant to glyphosate by one or more of these mechanisms. Previous studies by Ferreira and Reddy (2000) showed that epicuticular waxes can reduce glyphosate absorption in some broadleaf species such as coca [*Erythroxylum coca* var. *coca* (Lam.)]. Previous research with glyphosate-resistant rigid ryegrass, exhibiting 10-fold glyphosate resistance (Pratley et al. 1999), found that neither uptake, translocation, nor metabolism was responsible for the mechanism of resistance (Feng et al. 1999). Subsequent studies showed no evidence for gene amplification or cosegregation of a specific 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene variant with glyphosate resistance, suggesting that the resistance mechanism may be nontarget based (Baerson et al. 2002). Recent studies by Simarmata and Penner (2004) provided evidence that difference in sensitivity of EPSPS to glyphosate was a major contributor to glyphosate resistance in rigid ryegrass from California.

In horseweed, information on the mechanism of resistance to glyphosate is lacking. We understand that a research team in the Monsanto Company is aggressively investigating the mechanism of resistance in resistant (R) biotypes from several states (R. "Doug" Sammons, personal communication). Their research suggests that resistance to glyphosate is not due to reduced glyphosate uptake, glyphosate detoxification, or overexpression of EPSPS but due to reduced translocation (Feng et al. 2004). Recently, R biotypes of horseweed in Mississippi have been reported when glyphosate-resistant cotton (*Gossypium Herbaceum*) or soybean (*Gly-*

cine max) was grown for at least three consecutive growing seasons (Koger et al. 2004). In this article, we examined the involvement of absorption and translocation of glyphosate in the mechanism of resistance in horseweed biotypes from Mississippi. The specific objectives of the research were to (1) compare growth characteristics of R and susceptible (S) biotypes from Mississippi, Arkansas, Delaware, and Tennessee, (2) determine absorption and translocation of glyphosate in R and S biotypes, (3) compare epicuticular wax content for R and S biotypes, and (4) determine efficacy of leaf-treated glyphosate on whole plant control.

Materials and Methods

Seed Sources

Horseweed seeds were collected at maturity from field-grown plants in Mississippi (MS), Arkansas (AR), Tennessee (TN), and Delaware (DE). One R and one S biotype were collected from each state. Seeds of R biotypes from Tunica County, MS, Lawrence County, AR, and Haywood County, TN, were collected from plants that survived at least two applications of 0.84 kg ae ha^{-1} glyphosate in glyphosate-resistant cotton in 2003. Glyphosate-resistant cotton was grown in all three fields for at least three consecutive years. Seeds of an R biotype from Sussex County, DE, were collected from plants that survived at least two in-season 0.84 kg ae ha^{-1} applications of glyphosate in a no-till field that had been planted to glyphosate-resistant soybean for five consecutive years. S biotypes were collected from noncrop areas in the same counties as their respective R biotypes. Seeds were stored in separate screw-cap plastic bottles in the dark at 4 C until further use.

General Information

Seeds of each biotype were planted in the greenhouse in separate 26 by 52 by 6 cm trays containing a mixture of soil (Bosket sandy loam, fine-loamy, mixed thermic Molic Hapludalfs) and Jiffy Mix potting soil[1] (1:1, v/v). Seeds were spread on top of potting soil and subirrigated with distilled water. After emergence, seedlings in the cotyledon growth stage were transplanted to individual 11-cm-diam pots containing potting soil. Each treatment had 1 plant per pot. Plants were grown at 32/25 C (± 3 C) day/night temperature. Natural light was supplemented with light from sodium vapor lamps to provide a 14-h photoperiod. Plants were subirrigated as needed.

Leaf-dip Assay to Challenge Plants

Plants of the R and S biotypes from Mississippi were challenged with a leaf-dip glyphosate treatment assay. At the 10- to 13-leaf growth stage, one fully expanded leaf including the petiole was excised from each plant with a scalpel. The petiole along with bottom one-fourth of leaf was submerged inside a 7-ml plastic vial[2] containing 6.8 ml of glyphosate solution. Glyphosate solutions were prepared using a commercial formulation[3] of the potassium salt of glyphosate to give concentration of 0, 600, 1,200, 2,400, and 4,800 mg ae L^{-1} of glyphosate in double-distilled water. A nontreated check containing double-distilled water only was also included for each biotype. Vials were placed in a growth chamber maintained at 32/25 C day/night temperature with a 12-h photoperiod (200 μmol m^{-2} s^{-1}). Additional solution was added as needed to account for evaporation losses. Vials were removed from the growth chamber after 48 h and leaf injury was visually estimated based on severity of wilting and discoloration on a scale of 0 (no visual injury) to 10 (severe wilting and necrosis). Treatments were arranged in a completely randomized design. Each treatment was replicated four times and the experiment was repeated. Data were subjected to combined analysis of variance (ANOVA) and means were separated using Fisher's Protected LSD test at the 5% level of probability.

^{14}C-glyphosate Absorption and Translocation Study

Uniform plants having 23 to 29 leaves were selected for treatment with ^{14}C-glyphosate. Plant age, dry weight, and rosette diameter of plants at time of ^{14}C-glyphosate application are described in Table 3. The ^{14}C-glyphosate solution was prepared by diluting ^{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} as *N*-(phosponomethyl)glycine] in a commercial formulation of glyphosate[3] to give a final concentration of 0.84 kg ae ha^{-1} in 190 L of water (Reddy 2000). A 10-μl volume of the final ^{14}C-glyphosate solution containing 4.29 kBq was placed on the adaxial surface of a randomly selected youngest fully expanded leaf of the second whorl of leaves as 25, 3-mm-diam droplets. Plants were in the rosette growth stage and typically contained four whorls of leaves at the time of ^{14}C-glyphosate treatment. The first whorl (top) comprised the youngest leaves, which were not fully expanded at the time of treatment, and the fourth whorl (bottom) contained the oldest fully expanded leaves. Plants were not presprayed with commercial glyphosate before application of ^{14}C-glyphosate to minimize stress during the exposure period. Furthermore, pretreated (Camacho and Moshier 1991) and nontreated (Gillespie 1994) plants have produced similar absorption and translocation trends when radiolabeled herbicides were spotted on the leaves.

Plants were harvested at 48 h after ^{14}C-glyphosate treatment. The treated leaf including petiole was excised, and ^{14}C-glyphosate remaining on the leaf surface was removed by gently shaking for 20 s in 10 ml methanol–water (1:9, v/v) followed by an additional washing for 20 s in a second 10 ml methanol–water solution. Plants were sectioned into treated leaf, all other leaves, crown, and roots. Plant sections were wrapped in Kimwipes[4] tissue paper, placed in glass scintillation vials, and oven-dried at 40 C for 72 h. Oven-dried plant samples were combusted in a biological oxidizer,[5] and the evolved $^{14}CO_2$ was trapped in 10 ml Carbosorb E[6] and 10 ml Permafluor E^{+}[6]. Two 1-ml aliquots of each of the two leaf washes per treatment were mixed with 10 ml scintillation cocktail (EcoLume[7]). Radioactivity in leaf washes and oxidations were quantified using liquid scintillation spectrometry.[8]

The amount of ^{14}C present in the two leaf washes per treatment was summed. Amount of ^{14}C present in the leaf washes and plant sections was considered as total ^{14}C recovered, which averaged 99.8% of applied ^{14}C-glyphosate. Sum of the radioactivity present in all plant parts was con-

sidered as absorption and expressed as percentage of the ^{14}C recovered. Radioactivity present in all plant parts except the treated leaf was considered as translocated and expressed as a percentage of the ^{14}C absorbed. Treatments were arranged in a randomized complete block design. Each treatment was replicated five times and the experiment was repeated. Data were subjected to combined ANOVA and means were separated as described previously.

Wax Extraction

Two greenhouse-grown horseweed plants were selected on the basis of uniformity of rosette diameter and number of leaves. Plants were 163 d old and had 48 ± 6 leaves. Approximately 90 fully expanded leaves including petiole were excised from two plants and total fresh weight of leaves was recorded. Epicuticular wax was extracted using the procedure described previously by Chachalis et al. (2001). Wax was extracted by immersing leaves in 400 ml high-performance liquid chromatography grade chloroform in a glass beaker at room temperature for 20 s in a sonicator.[9] The chloroform–wax solution was filtered using a fritted glass funnel apparatus with Durapore[10] membrane filters (0.22 μm GV), and the volume was reduced to approximately 10 ml in a rotary evaporator.[11] The reduced chloroform–wax solution was transferred to a preweighed 20-ml glass scintillation vial. Chloroform was evaporated to dryness in a forced-air oven at 40 C for 72 h. The leaf surface area was determined after washing with chloroform using a stationary leaf area meter.[12] Wax mass was expressed as wax mass per unit leaf area and wax mass per unit leaf fresh weight. Three replicates of each R and S biotype were analyzed. The experiment was repeated. The Tennessee-susceptible (TN-S) biotype was not included for lack of plants.

Efficacy of Leaf-treated Glyphosate on Whole Plant Study

Plants in the rosette growth stage having 25 to 29 leaves were selected for treatment with glyphosate. The glyphosate solution was prepared using a commercial formulation[3] at 0.84 kg ae ha^{-1} (1× field rate) in 190 L of double-distilled water. Two fully expanded leaves of the second whorl were randomly selected for treatment. Ten microliter of glyphosate solution was placed on the adaxial surface of each leaf as 20 droplets. Droplets were placed between veins and not on the leaf petiole or midrib. A nontreated check for each biotype was included. The TN-S biotype was not included in the experiment. Fresh weight of live plant biomass was recorded at 3 wk after treatment (WAT). Data were expressed as percent fresh weight reduction as compared with the nontreated check. Treatments were arranged in a randomized complete block design. Treatments were replicated four times and the experiment was repeated. Data were subjected to combined ANOVA and means separation test as described previously.

Results and Discussion

Leaf-dip Assay and Confirmation of Resistance and Susceptibility of Test Plants

It was critical to confirm resistance and susceptibility of horseweed biotypes before they were used in further studies because seeds were collected from different fields and plants. We developed a leaf-dip assay to challenge plants without destruction of plants. Differences in leaf injury between R and S plants decreased with increased glyphosate rate (Table 1). Glyphosate at 600 mg ae L^{-1} resulted in a wider window of difference between leaf injury for R and S plants. Leaf injury symptoms were primarily wilting and necrotic spots on the intervenous portions of the leaf and necrosis around the leaf perimeter. These results indicate that R and S plants can be discriminated using glyphosate at a concentration of 600 mg ae L^{-1}. The single leaf-dip assay used was rapid, simple, nondestructive, and eliminated stress on plants. This leaf-dip assay allowed confirmation of both R and S plants simultaneously compared with a conventional method of selection of survivors as R plants 2 to 3 wk after 1× glyphosate treatment. However, the glyphosate treatment method is not suitable for S plants because glyphosate is lethal to S plants.

Table 1. Effect of glyphosate concentration on single leaf from glyphosate-resistant and glyphosate-susceptible horseweed plants from Mississippi, dipped in glyphosate solution for 48 h.[a,b,c]

Glyphosate concentration	Visual leaf injury[d]	
	R	S
mg ae L^{-1}	no.	
0	0	0
600	1	6
1,200	6	8
2,400	8	10
4,800	9	10
LSD (0.05)	1	

[a] Abbreviations: R, resistant; S, susceptible.
[b] Plants were maintained in the greenhouse at 32/25 C day/night temperature, with a 14-h photoperiod.
[c] Single leaf from each plant was excised and the petiole along with bottom one-fourth of leaf was submerged inside a 7-ml plastic vial containing glyphosate solution in a growth chamber maintained at 32/25 C day/night temperature with a 12-h photoperiod (200 μmol m^{-2} s^{-1}).
[d] Leaf injury was visually estimated based on severity of wilting and discoloration on a scale of 0 (no visual injury) to 10 (severe wilting, or discoloration, or both).

The R and S plants of all biotypes used in the studies were challenged using the leaf-dip assay described above to confirm resistance and susceptibility. Plants at the 10- to 13-leaf growth stage were challenged using 600 mg ae L^{-1} rate of glyphosate. The number of plants tested and the mean leaf injury rating for each biotype are shown in Table 2. On the basis of leaf injury score, plants were classified as susceptible if the score was 5 to 6 and resistant if the score was 2 to 3. The challenged plants were allowed to grow in the greenhouse until used in studies.

Growth Characteristics of R and S Biotypes

Horseweed plants used in the ^{14}C-glyphosate study were 136 d old and had a total of 23 to 29 (young and mature) leaves (Table 3). There were no differences in number of leaves between R and S biotypes from within and among the four states. Delaware-susceptible biotype had the highest rosette diameter, TN-S biotype had the smallest rosette diameter, and other biotypes had intermediate rosette diameter. A trend similar to rosette diameter was observed in plant

TABLE 2. Injury to single leaf of glyphosate-resistant and glyphosate-susceptible horseweed plants from Mississippi, Arkansas, Tennessee, and Delaware, after dipping in 600 mg ae L^{-1} glyphosate solution for 48 h.[a,b,c]

State	Number of plants tested		Visual leaf injury[d]	
	R	S	R	S
	no.			
Mississippi	152	159	2	6
Arkansas	151	159	2	6
Tennessee	152	67	3	5
Delaware	152	159	2	6
LSD (0.05)			0.5	

[a] Abbreviations: R, resistant; S, susceptible.
[b] Plants were maintained in the greenhouse at 32/25 C day/night temperature, with a 14-h photoperiod.
[c] Single leaf from each plant was excised and the petiole along with bottom one-fourth of leaf was submerged inside a 7-ml plastic vial containing glyphosate solution in a growth chamber maintained at 32/25 C day/night temperature with a 12-h photoperiod (200 μmol m^{-2} s^{-1}).
[d] Leaf injury was visually estimated based on severity of wilting and discoloration on a scale of 0 (no visual injury) to 10 (severe wilting, or discoloration, or both).

(shoot and root) dry weight. Plant dry weight was higher in R vs. S biotype from Tennessee, lower in R vs. S biotype from Delaware and Mississippi, and similar in R vs. S biotype from Arkansas. On the basis of the plant dry weight of R and S biotypes, the plant size decreased in the order: Delaware > Mississippi > Arkansas ≥ Tennessee.

^{14}C-glyphosate Absorption and Translocation

At 48 h after treatment, absorption of ^{14}C-glyphosate was similar between R and S biotypes from within and among the four states (Figure 1). Absorption of ^{14}C-glyphosate ranged from 47 to 54% among the eight biotypes. Similarly, Feng et al. (1999) observed nearly identical uptake of ^{14}C-glyphosate in glyphosate-resistant and glyphosate-susceptible biotypes of rigid ryegrass from Australia. Overall, absorption of ^{14}C-glyphosate in horseweed was lower than that reported for rigid ryegrass (≈ 70%) by Feng et al. (1999).

The amount of ^{14}C-glyphosate translocated from the treated leaf to other plant parts ranged from 16 to 20% in R biotypes and 28 to 31% in S biotypes at 48 h after treatment (Table 4). Overall, the reduction in translocation of

TABLE 3. Growth characteristics of glyphosate-resistant and glyphosate-susceptible horseweed biotypes from Mississippi, Arkansas, Tennessee, and Delaware, at the time of ^{14}C-glyphosate application.[a]

Biotype[b]	Plant age	Plant dry weight[c]	Rosette diameter	Leaves
	d	mg $plant^{-1}$	mm	no. $plant^{-1}$
MS-R	136	340	112	26
MS-S	136	472	125	27
AR-R	136	315	110	23
AR-S	136	263	100	23
TN-R	136	322	112	24
TN-S	136	213	75	23
DE-R	136	450	120	25
DE-S	136	523	147	29
LSD (0.05)	—	64	18	NS

[a] Plants were grown in the greenhouse before and after ^{14}C-glyphosate application. Greenhouse was maintained at 32/25 C day/night temperature, with a 14-h photoperiod.
[b] Abbreviations: AR-R, Arkansas resistant; AR-S, Arkansas susceptible; DE-R, Delaware resistant; DE-S, Delaware susceptible; MS-R, Mississippi resistant; MS-S, Mississippi susceptible; TN-R, Tennessee resistant; TN-S, Tennessee susceptible; NS, not significant.
[c] Sum of the dry weight of leaves, crown, and roots.

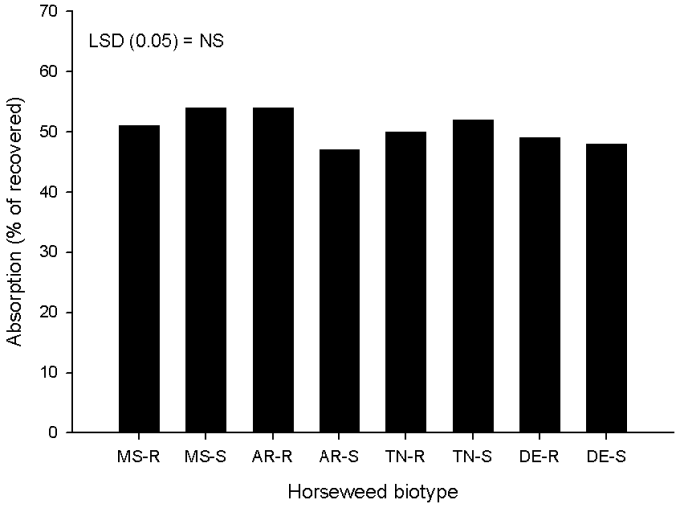

FIGURE 1. ^{14}C-glyphosate absorption in glyphosate-resistant and glyphosate-susceptible horseweed biotypes from Mississippi, Arkansas, Tennessee, and Delaware at 48 h after treatment. Abbreviations: AR-R, Arkansas resistant; AR-S, Arkansas susceptible; DE-R, Delaware resistant; DE-S, Delaware susceptible; MS-R, Mississippi resistant; MS-S, Mississippi susceptible; TN-R, Tennessee resistant; TN-S, Tennessee susceptible. Plants were grown in the greenhouse before and after ^{14}C-glyphosate application. Greenhouse was maintained at 32/25 C day/night temperature, with a 14-h photoperiod.

TABLE 4. ^{14}C-glyphosate translocation and distribution in glyphosate-resistant and glyphosate-susceptible horseweed biotypes from Mississippi, Arkansas, Tennessee, and Delaware, at 48 h after treatment.[a]

Biotype[b]	Translocation[c]	^{14}C-glyphosate distribution[d]			
		All other leaves	Crown	Roots	Treated leaf
	% of absorbed				
MS-R	20.3	6.8	0.9	12.6	79.7
MS-S	28.1	7.9	1.6	18.6	71.9
AR-R	18.7	6.1	0.8	11.8	81.3
AR-S	30.2	8.9	1.8	19.5	69.8
TN-R	17.1	4.9	0.9	11.3	82.9
TN-S	29.4	6.8	1.2	21.4	70.6
DE-R	16.4	3.9	0.6	11.9	83.6
DE-S	31.3	8.3	1.5	21.5	68.7
LSD (0.05)	2.0	1.5	0.3	1.6	2.1

[a] Plants were grown in the greenhouse before and after ^{14}C-glyphosate application. Greenhouse was maintained at 32/25 C day/night temperature, with a 14-h photoperiod.
[b] Abbreviations: AR-R, Arkansas resistant; AR-S, Arkansas susceptible; DE-R, Delaware resistant; DE-S, Delaware susceptible; MS-R, Mississippi resistant; MS-S, Mississippi susceptible; TN-R, Tennessee resistant; TN-S, Tennessee susceptible.
[c] ^{14}C-glyphosate outside of treated leaf (other leaves, crown, and roots) is considered as translocation.
[d] ^{14}C-glyphosate distribution throughout the plant is based on percent of ^{14}C-glyphosate absorbed by 48 h after treatment.

TABLE 5. ^{14}C-glyphosate concentration in treated leaf, other leaves, crown, and roots of glyphosate-resistant and glyphosate-susceptible horseweed biotypes from Mississippi, Arkansas, Tennessee, and Delaware, at 48 h after treatment.[a]

Biotype[b]	Plant portion[c]			
	Treated leaf	Other leaves	Crown	Roots
	ng ^{14}C-glyphosate g^{-1} tissue dry weight			
MS-R	9,874	48	158	404
MS-S	8,031	71	240	770
AR-R	11,634	65	109	408
AR-S	7,758	97	293	1,051
TN-R	14,349	46	132	348
TN-S	12,904	81	253	796
DE-R	9,229	26	143	211
DE-S	7,589	47	272	409
LSD (0.05)	1,069	19	76	180

[a] Plants were grown in the greenhouse before and after ^{14}C-glyphosate application. Greenhouse was maintained at 32/25 C day/night temperature, with a 14-h photoperiod.

[b] Abbreviations: AR-R, Arkansas resistant; AR-S, Arkansas susceptible; DE-R, Delaware resistant; DE-S, Delaware susceptible; MS-R, Mississippi resistant; MS-S, Mississippi susceptible; TN-R, Tennessee resistant; TN-S, Tennessee susceptible.

[c] ^{14}C-glyphosate distribution throughout plant is based on percent of ^{14}C-glyphosate absorbed by 48 h after treatment.

^{14}C-glyphosate ranged from 28% of absorbed in Mississippi-R biotype to 48% in Delaware-R (DE-R) biotype compared with their respective S biotypes. Unlike absorption, there was measurable decrease in translocation of ^{14}C-glyphosate in R biotypes compared with S biotypes.

Radioactivity was distributed throughout the plant with ^{14}C accumulation decreasing in the order, treated leaf > roots > other leaves > crown regardless of biotypes. More radioactivity was retained in treated leaf of R biotype compared with their respective S biotype. Conversely, less radioactivity was accumulated in other leaves, crown, and roots of R biotype compared with their respective S biotype. Because of the variability in plant weight among biotypes, the radioactivity distribution data were expressed as concentration (nanogram of ^{14}C-glyphosate per unit plant tissue) to normalize the data for plant weight (Table 5). Again, the pattern of ^{14}C-glyphosate concentration in plant parts was nearly identical to the ^{14}C-glyphosate accumulation expressed as percent of absorbed. To be effective, lethal amounts of glyphosate must reach its target site from the site of application (Duke 1988). Because absorption of glyphosate was similar between R and S biotypes, the loss of efficacy in R biotypes could be attributed to sequestration of glyphosate in a metabolically inactive site. The crown, with young and mature leaves, and roots are centers of high metabolic activity. Consequently, decreased radioactivity accumulation in these plant parts of R biotype compared with S biotype may in part explain the mechanism of resistance in horseweed. Reduced glyphosate translocation has been implicated as the mechanism of resistance in other horseweed R biotypes from Tennessee and Delaware (Doug Sammons, personal communication).

Leaf Wax Mass

Total amount of wax found on horseweed biotypes varied from 6 to 80 μg cm^{-2} (Table 6). There were no differences in wax content between R and S biotypes regardless of origin. The lowest amount was from Arkansas-S biotype and the highest was from DE-R biotype. The wax was expressed per unit leaf weight basis to normalize the wax data for leaf weight because of the variability in leaf size and leaf weight among biotypes. The wax per unit leaf weight data also followed the trend similar to wax per unit leaf area with no differences between R and S biotypes regardless of origin. The epicuticular wax mass in most plant species varies from 10 to 200 μg cm^{-2} (McWhorter 1993). However, the wax mass observed in these R and S horseweed biotypes is similar to the levels (14 to 57 μg cm^{-2}) observed in ivyleaf morningglory [*Ipomoea hederacea* (L.) Jacq.] and smallflower morningglory [*Jacquemontia tamnifolia* (L.) Griseb.] (Chachalis et al. 2001). Epicuticular wax has been considered as the main barrier to herbicide absorption. Removal of wax from coca leaves increased glyphosate absorption indicating that epicuticular wax could act as a barrier against ^{14}C-glyphosate absorption (Ferreira and Reddy 2000). In this study, lack of differences in glyphosate absorption may have been due to similar amounts of wax on leaves of R and S biotypes within a state. Because both R and S biotypes from each state had similar amounts of leaf wax and absorption of ^{14}C-glyphosate, the differential sensitivities to glyphosate between R and S biotypes may have been caused by differences in translocation.

TABLE 6. Epicuticular wax content for glyphosate-resistant and glyphosate-susceptible horseweed biotypes from Mississippi, Arkansas, Tennessee, and Delaware.[a]

Biotype[b]	Epicuticular wax content	
	μg wax cm^{-2} leaf area	μg wax g^{-1} fresh weight
MS-R	67	2,257
MS-S	72	2,727
AR-R	10	346
AR-S	6	191
TN-R	72	2,947
TN-S	—[c]	—[c]
DE-R	80	3,097
DE-S	62	2,506
LSD (0.05)	20	856

[a] Plants were maintained in the greenhouse at 32/25 C day/night temperature, with a 14-h photoperiod.

[b] Abbreviations: AR-R, Arkansas resistant; AR-S, Arkansas susceptible; DE-R, Delaware resistant; DE-S, Delaware susceptible; MS-R, Mississippi resistant; MS-S, Mississippi susceptible; TN-R, Tennessee resistant; TN-S, Tennessee susceptible.

[c] TN-S biotype was not included.

Efficacy of Leaf-treated Glyphosate on Whole Plant

Treating two leaves with 1× glyphosate solution killed S plants from Mississippi, Arkansas, and Delaware (Table 7). In contrast to S plants, treating two leaves of R plants resulted in 38 to 58% control. The loss of herbicide efficacy in R plants was mainly due to reduction in translocation of glyphosate. This efficacy data supports reduced translocation observed in ^{14}C-glyphosate study (Table 4). The efficacy data also suggest that increasing the rate of glyphosate would increase control of an R biotype.

There were differences in growth characteristics of horse-

TABLE 7. Shoot fresh weight reduction of glyphosate-resistant and glyphosate-susceptible horseweed biotypes from Mississippi, Arkansas, Tennessee, and Delaware at 3 wk after glyphosate treatment.[a,b,c]

State	Shoot fresh weight reduction	
	R	S
	%L[d]	
Mississippi	38	100
Arkansas	58	100
Tennessee	47	—[e]
Delaware	50	100
LSD (0.05)	15	

[a] Abbreviations: R, resistant; S, susceptible.
[b] Ten microliter of glyphosate solution (0.84 kg ae ha^{-1} in 190 L water) was applied as 20 droplets to the adaxial surface of two leaves of each plant.
[c] Plants were maintained in the greenhouse at 32/25 C day/night temperature, with a 14-h photoperiod.
[d] As compared with the fresh weight of nontreated check for each R and S biotype.
[e] TN-S biotype was not included.

weed biotypes. On the basis of the plant dry weight of R and S biotypes, the plant size decreased in the order: Delaware > Mississippi > Arkansas ≥ Tennessee. The nondestructive bioassay proved to be useful for confirming R and S test plants and may have potential for identifying R populations in grower's fields. Absorption of ^{14}C-glyphosate was similar (47 to 54%) between R and S plants from within and among the four states, suggesting absorption is not involved in glyphosate resistance. The amount of radioactivity translocated from the treated leaf was reduced in R plants compared with S plants and the reduction ranged from 28 to 48% of absorbed. Radioactivity was distributed throughout the plant, but less radioactivity was accumulated in other leaves, crown, and roots of R biotype compared with respective S biotype. Total amount of leaf wax found on horseweed biotypes varied from 6 to 80 μg cm^{-2} with no differences in wax content between R and S biotypes regardless of origin. Treating two leaves with glyphosate solution at field use rate (0.84 kg ha^{-1}) killed the S biotype but not the R biotype (38 to 58% control) regardless of state origin. These results suggest that reduced translocation of glyphosate plays a major role in glyphosate resistance in R biotype of horseweed. However, further elucidation of causes for reduced translocation and potential involvement of other mechanisms such as sequestration of glyphosate, glyphosate degradation, sensitivity of EPSPS enzyme, and leaf surface characteristics of R and S biotypes is currently under investigation.

Sources of Materials

[1] Jiffy mix, Jiffy Products of America Inc., 951 Swanson Drive, Batavia, IL 60510.
[2] High-density polyethylene vial, Fisher Scientific, Liberty Lane, Hampton, NH 03842.
[3] Potassium salt of glyphosate, Roundup WEATHERMAX™, Monsanto Company, 800 North Linbergh Boulevard, St. Louis, MO 63167.
[4] Kimwipes EX-L, Kimberly-Clark Corporation, 1400 Holcomb Bridge Road, Roswell, GA 30076.
[5] Packard Oxidizer 306, Packard Instruments Company, 2200 Warrenville Road, Downers Grove, IL 60515.
[6] Carbosorb E and Permafluor E^+, Packard BioScience Company, 800 Research Parkway, Meridian, CT 06450.
[7] EcoLume, ICN, 330 Hyland Avenue, Costa Mesa, CA 92626.
[8] Tri-carb 2500TR Liquid Scintillation Analyzer, Packard BioScience Company, 800 Research Parkway, Downers Grove, IL 60515.
[9] Branson 2210 Sonicator, Branson Ultrasonics Corporation, 41 Eagle Road, Danbury, CT 06813-1961.
[10] Durapore Membrane filters, Millipore Corporation, 80 Ashby Road, Bedford, MA 01730.
[11] Buchi R-124 Rotavapor, Buchi Analytical Inc., 19 Lukens Drive, NewCastle, DE 19720.
[12] Leaf Area Meter, LI-3100, LI-COR Inc., 4421 Superior Street, Lincoln, NE 68501.

Acknowledgments

We thank Efren Ford and Albert Tidwell for technical assistance. We also extend our thanks to Bill Barksdale, Eric Palmer, and Mark VanGessel for their assistance in providing seed used for this research. We are also grateful to Greg Ferguson, Monsanto Company, St. Louis, MO, for supplying ^{14}C-glyphosate.

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Received June 4, 2004, and approved September 23, 2004.