

# Absorption and translocation of glyphosate in glyphosate-resistant cotton as influenced by application method and growth stage

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The influence of herbicide placement and plant growth stage on the absorption and translocation patterns of  $^{14}\text{C}$ -glyphosate in glyphosate-resistant cotton was investigated. Plants at four growth stages were treated with  $^{14}\text{C}$ -glyphosate on a 5-cm<sup>2</sup> section of the stem, which simulated a postemergence-directed spray (PDS) application, or on the newest mature leaf, which simulated a postemergence (POST) application. Plants were harvested 3 and 7 d after treatment and divided into the treated leaf or treated stem, mature leaves, immature leaves and buds, stems, roots, fruiting branches (including the foliage on the fruiting branch), squares, and bolls. The PDS versus POST application main effect on absorption was significant. Absorption of  $^{14}\text{C}$ -glyphosate applied to stem tissue was higher in PDS applications than in POST applications. Plants receiving PDS applications absorbed 35% of applied  $^{14}\text{C}$ -glyphosate, whereas those receiving POST applications absorbed 26%, averaged over growth stages at application. Absorption increased from the four-leaf growth stage to the eight-leaf stage in POST applications but reached a plateau at the eight-leaf stage. Plants with PDS applications showed an increase in absorption from the four- to eight- to twelve-leaf stages and reached a plateau at the 12-leaf stage. Translocation of  $^{14}\text{C}$ -glyphosate to roots was greater at all growth stages with PDS treatments than with POST treatments. Herbicide placement did not affect translocation of  $^{14}\text{C}$ -glyphosate to squares and bolls. Squares and bolls retained 0.2 to 3.7% of applied  $^{14}\text{C}$ -glyphosate, depending on growth stage. Separate studies were conducted to investigate the fate of foliar-applied  $^{14}\text{C}$ -glyphosate at the four- or eight-leaf growth stages when harvested at 8- or 10-leaf, 12-leaf, midbloom (8 to 10 nodes above white bloom), and cutout (five nodes above white bloom, physiological maturity) stages. Thirty to 37% of applied  $^{14}\text{C}$ -glyphosate remained in the plant at cutout in four- and eight-leaf treatment stages, respectively. The concentration of  $^{14}\text{C}$ -glyphosate in tissue (Bq g<sup>-1</sup> dry weight basis) was greatest in mature leaves and immature leaves and buds in plants treated at the four-leaf stage. Plants treated at the eight-leaf stage and harvested at all growth stages except cutout showed a higher concentration of  $^{14}\text{C}$ -glyphosate in squares than in other plant tissue. Accumulation of  $^{14}\text{C}$ -glyphosate in squares reached a maximum of 43 Bq g<sup>-1</sup> dry weight at harvest at the 12-leaf stage. This concentration corresponds to 5.7 times greater accumulation of  $^{14}\text{C}$ -glyphosate in squares than in roots, which may also be metabolic sinks. These data suggest that reproductive tissues such as bolls and squares can accumulate  $^{14}\text{C}$ -glyphosate at higher concentrations than other tissues, especially when the herbicide treatment is applied either POST or PDS during reproductive stages (eight-leaf stage and beyond).

**Nomenclature:** Glyphosate; cotton, *Gossypium hirsutum* L. 'Delta Pine 5415RR'.

**Key words:** Herbicide-resistant crops, transgenic crops.

Glyphosate was registered for use in glyphosate-resistant cotton in the United States in 1997. A naturally occurring 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene [E.C. 2.5.1.19], identified from *Agrobacterium* sp. strain CP4, whose protein product is glyphosate resistant (Barry et al. 1992; Padgett et al. 1995) was cloned from and expressed in several crop plants, including cotton (Nida et al. 1996).

Certain restrictions are specified in the glyphosate use registration for glyphosate-resistant cotton (Anonymous 1999). Producers may make foliar postemergence (POST) applications to cotton through the four-leaf stage of crop development. Beyond this stage of crop growth, producers are restricted to POST-directed spray (PDS) applications to

minimize glyphosate contact with leaf tissue. Producers may apply a maximum of two POST and two PDS applications of 1.12 kg ha<sup>-1</sup> each growing season. Sequential glyphosate applications must be at least 10 d apart, and cotton must have at least two nodes of incremental growth between applications (Anonymous 1999). These restrictions on glyphosate application are not required for other CP4-EPSPS-containing glyphosate-resistant crops such as soybean [*Glycine max* (L.) Merr.]. A lower tolerance for glyphosate in glyphosate-resistant cotton compared to other glyphosate-resistant crops could be a reason for the differences in use restrictions.

Since its commercial availability, there have been performance and yield loss complaints on glyphosate-resistant cot-

ton in several southeastern states because of a wide-spread, but not rigorously documented, increase in lower fruiting branch boll abortion and misshapen bolls (Ferreira et al. 1998; Vargas et al. 1998). These symptoms typically occur on the first and second fruiting positions of the lower fruiting branches of glyphosate-treated cotton. Yields are often not affected by these early-season losses because cotton compensates by relocating the boll load higher and to further fruiting positions on the plant, than on nontreated plants (Kalahaer and Coble 1998). However, this late-season compensation can delay harvest and cause yield loss if the season is not long enough for compensatory growth (Jones and Snipes 1999).

Nida et al. (1996) confirmed CP4-EPSPS expression in leaf and seed samples of two early transformed lines of cotton by enzyme-linked immunosorbent assay (ELISA) analysis. However, there are no reports available to document whether the CP4-EPSPS enzyme is being expressed sufficiently to prevent glyphosate injury in the floral structures of glyphosate-resistant cotton. Foliar applications of glyphosate beyond the four-leaf stage of cotton growth can result in injury to cotton reproductive structures. Kalahaer and Coble (1998) found a significant seed-cotton yield reduction when glyphosate was applied POST to cotton at the eight-leaf growth stage and the first-white-bloom stage, indicating a greater glyphosate sensitivity during reproductive development than during vegetative stages. If the CP4-EPSPS enzyme is not being expressed in key reproductive tissues and if the glyphosate that remains in the plant accumulates in these tissues, pollen production or pollination may be affected. A significant reduction of pollen viability in glyphosate-resistant cotton treated with glyphosate at the four-leaf stage (POST) and eight-leaf stage (PDS) was reported at 1–2 wk after first bloom (WAFB) in greenhouse studies and 1–3 WAFB in field studies by Pline et al. (2001).

The position or placement of a glyphosate application has been reported to affect glyphosate absorption significantly. Wills (1978) found five- to sevenfold greater glyphosate toxicity to nonglyphosate-resistant cotton when glyphosate was applied to the lower stem portion of the plant than when applied to the first true leaf. Translocation data confirmed that movement of  $^{14}\text{C}$ -glyphosate was significantly greater following treatment to the mature lower stem than to the mature lower leaves or to immature upper stem or leaves of cotton. These data suggest that PDS treatments, as mandated by the glyphosate label after the four-leaf stage, may still pose a risk to cotton because of the greater potential for glyphosate absorption and translocation via lower stem entry. Because sufficient CP4-EPSPS expression occurs in leaves (Nida et al. 1996), this increase in absorption or translocation would only be detrimental if glyphosate accumulation in certain tissues surpasses a threshold level, overwhelming the resistance mechanism and causing toxicity. In addition, the glyphosate threshold for specific plant functions such as pollination or floral development may be lower than the threshold level for more general aspects of development.

Several studies have reported that glyphosate distribution parallels that of photoassimilates in a variety of plants (Gougler and Geiger 1981; McAllister and Haderlie 1985), thus generally following a source-to-sink relationship (Sand-

berg et al. 1980; Wyrill and Burnside 1976). Therefore, the potential exists for glyphosate to accumulate in developing flowers and bolls because they serve as metabolic sinks during cotton development.

Growth stage has been shown to affect absorption and translocation of  $^{14}\text{C}$ -glyphosate in both weeds and crops (Davis et al. 1979; Tardiff and Leroux 1991). There appears to be no direct relationship of growth stage and glyphosate behavior; instead, the effect of plant growth stage on glyphosate movement seems to be species dependent. Mature soybean leaves absorb more glyphosate than immature leaves (McWhorter et al. 1980), whereas younger johnsongrass [*Sorghum halepense* (L.) Pers.] plants absorb more glyphosate than mature plants (Camacho and Moshier 1991). Likewise, the growth stage of glyphosate-resistant cotton at the time of application may influence glyphosate absorption and translocation and possibly crop tolerance.

The first objective of this research was to determine whether foliar or stem application position influences absorption or translocation of glyphosate in 4-, 8-, and 12-leaf and 2 WAFB cotton. A second objective was to evaluate the fate of glyphosate foliar-applied to four- and eight-leaf cotton as assessed at four intervals of cotton growth and reproduction.

## Materials and Methods

### Plant Material and Growth Conditions

DeltaPine 5415RR glyphosate-resistant cotton was planted in 30-cm pots containing Metro-Mix 360<sup>1</sup> and grown in a plastic greenhouse maintained at  $25 \pm 2$  C constant temperature where natural sunlight was supplemented 4 h daily with mercury halide lights, providing a 16-h day length. Treatment placement studies were conducted from September 1999 to January 2000, and distribution of  $^{14}\text{C}$ -glyphosate through the cotton life cycle studies were conducted from October 1999 to March 2000, with all plants in each run for each study being planted on the same date. Applications of  $^{14}\text{C}$ -glyphosate were made as plants reached their respective treatment growth stage (treatments were not all made on the same date).

### $^{14}\text{C}$ -Glyphosate Treatments and Sampling

For treatment position studies, plants were treated with  $^{14}\text{C}$ -glyphosate<sup>2</sup> at the 4-leaf (vegetative stage), 8-leaf (early square formation, reproductive stage) 12-leaf (squares visible, reproductive stage), and 2 WAFB (squares, blooms, and bolls present, reproductive stage) growth stages. At each growth stage, the uppermost fully expanded main stem leaf or the stem was treated.  $^{14}\text{C}$ -glyphosate was applied to plants on either a 5 by 1 cm strip of the leaf (directly over and aligned with the midvein, POST treatments) or the stem (starting at 2 cm above the soil line and continuing to 7 cm above the soil line, PDS treatments). For glyphosate fate studies,  $^{14}\text{C}$ -glyphosate was applied only to leaf tissue (newest main stem mature leaf) at the four- and eight-leaf stages in the same manner as described above. In both studies, a microsyringe equipped to deliver 1- $\mu\text{l}$  droplets was used to evenly apply 10, 1- $\mu\text{l}$  droplets of  $^{14}\text{C}$ -glyphosate plus 0.25% (v/v) nonionic surfactant<sup>3</sup> in water containing a total of 5,000 Bq [specific activity of  $^{14}\text{C}$ -glyphosate was

88.8 kBq  $\mu\text{mol}^{-1}$ ]. The droplets were evenly spread over the marked 5-cm<sup>2</sup> surface area on either the leaf or stem in order to compare absorption over an equal area. This rate of <sup>14</sup>C-glyphosate is equal to 190 g ai ha<sup>-1</sup>, which is about 17% of the recommended field use rate of 1.12 kg ai ha<sup>-1</sup> glyphosate.

Plants were harvested either 3 or 7 d after treatment (DAT) for treatment position studies or at the 8-leaf, 12-leaf, midbloom, or cutout growth stages for glyphosate fate studies. At harvest, the treated leaf or stem was removed and rinsed with 10 ml of 1:1 water : methanol plus 0.25% (v/v) nonionic surfactant.<sup>3</sup> A 1-ml subsample from each leaf/stem rinse was counted using liquid scintillation spectrometry to determine the amount of nonabsorbed herbicide. Treated plants were divided into the following parts: treated leaf or stem, mature leaves, immature leaves and buds, untreated stem, roots, fruiting branches and its foliage, squares, and bolls. Plant parts were dried by forced air at 50 C, and dry weights were recorded. Plant samples were ground to a homogeneous mixture in a coffee grinder<sup>4</sup> and 100-mg subsamples were combusted using a Harvey biological oxidizer<sup>5</sup> to recover absorbed <sup>14</sup>C-glyphosate as <sup>14</sup>CO<sub>2</sub>. Accuracy of subsampling was  $\geq 93\%$ . Recovered radioactivity was quantified by liquid scintillation spectrometry, and the background reading was subtracted from all data points. Foliar or stem absorption was calculated as a percentage of the total applied, and total absorption was calculated as the sum of all <sup>14</sup>C recovered from oxidized plant parts. Distribution of <sup>14</sup>C in plant tissues was expressed as a percentage of applied radioactivity or as Bq g<sup>-1</sup> of tissue dry weight. Recovery of <sup>14</sup>C in treatment placement studies averaged 89%, whereas recovery for glyphosate distribution throughout the cotton life cycle studies ranged from 36.1 to 99.5%, primarily as a function of the days between treatment and harvest.

Metabolism of <sup>14</sup>C-glyphosate was not investigated because of reports of negligible metabolism by plants (Duke 1988). Therefore, <sup>14</sup>C present in plant tissue was assumed to be <sup>14</sup>C-glyphosate, and that term will be used in this work.

## Experimental Design and Data Analysis

The glyphosate application position study was arranged as a three-factor (treatment placement by plant growth stage by harvest interval) factorial in a completely randomized design with four replications and was repeated. The glyphosate fate study was arranged as a two-factor (growth stage at treatment by growth stage at harvest) factorial in a completely randomized design with four replications and was repeated. Analysis of variance (ANOVA) conducted using SAS<sup>6</sup> revealed no run by treatment interactions, so data were combined over runs. Absorption and translocation data were analyzed for main effects and interactions. Residuals were plotted, and logarithmic transformations were conducted on data where variance increased with increasing means. Following ANOVA, treatment or log-transformed treatment means were compared using Fisher's protected LSD test at the 5% probability level.

## Results and Discussion

### Treatment Placement and Growth Stage Effects on Absorption of <sup>14</sup>C-Glyphosate

Absorption of <sup>14</sup>C-glyphosate by cotton was not influenced by harvest interval, with harvests at 3 and 7 DAT

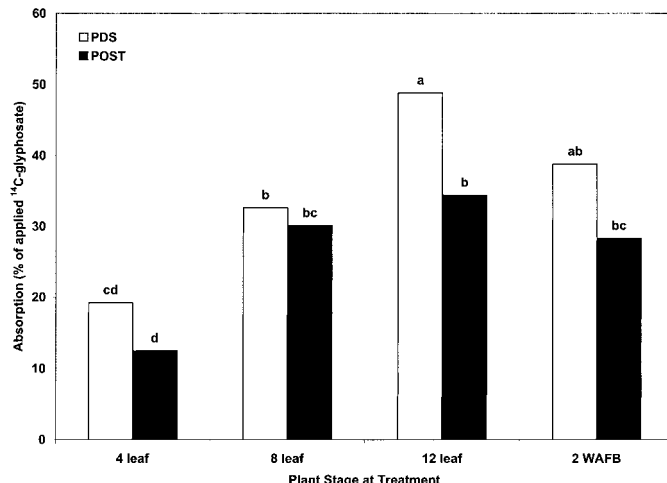


FIGURE 1. Absorption of <sup>14</sup>C-glyphosate averaged over 3 and 7 d after treatment in glyphosate-resistant Delta Pine 5415RR cotton (*Gossypium hirsutum*) applied to a 5-cm<sup>2</sup> area of either the stem (postemergence-directed) or the newest mature leaf (postemergence) at the 4-, 8-, and 12-leaf stage or at 2 wk after first bloom. Absorption values are expressed as a percentage of applied <sup>14</sup>C-glyphosate. Means were separated with Fisher's protected LSD at  $\alpha = 0.05$ . Means with the same letter are not significantly different from each other.

having similar absorption means (data not shown). Absorption data from both harvest intervals were thus combined. These data suggest that absorption of <sup>14</sup>C-glyphosate 3 DAT had reached a plateau. Absorption of foliar-applied glyphosate has previously been described as biphasic, with initial rapid glyphosate absorption in the first 24 h followed by a longer phase of slow uptake (Gaskin and Holloway 1992; Masiunas and Weller 1988).

The main effects of treatment placement and growth stage at application were both significant at  $\alpha = 0.05$ , but their interaction was not significant. <sup>14</sup>C-glyphosate absorption in plants receiving PDS treatments was significantly greater than in those receiving POST treatments (Figure 1). Plants receiving PDS applications absorbed 35% of applied <sup>14</sup>C-glyphosate, whereas those receiving POST applications absorbed 26%, averaged over growth stages, at application. Wills (1978) also reported greater glyphosate absorption through stem tissue than leaf tissue in nontransgenic cotton. Therefore on an equal area basis, <sup>14</sup>C-glyphosate absorption through stem tissue appears greater than through leaf tissue. However, on a whole-plant basis, the actual amount of glyphosate absorbed through the lower stem may be less in field applications than absorption through leaf tissue because the stem comprises less total surface area than leaves (Reynolds 2000). These differences in total surface area may outweigh differences in application position in commercial field applications.

The amount of <sup>14</sup>C-glyphosate absorption was highly dependent on the growth stage of the plant at the time of treatment ( $P < 0.0001$ ). Absorption of <sup>14</sup>C-glyphosate averaged over treatment placements was 19% of that applied at the four-leaf stage and increased to 29, 45, and 41% of that applied at the 8-leaf, 12-leaf, and the midbloom stages, respectively (Figure 1). Harris and Vencill (1999) found that <sup>14</sup>C-glyphosate absorption in cotton at the match-head square growth stage (after the four-leaf stage but before the eight-leaf stage) was approximately twice that of glyphosate

applied at the first white flower stage (after the 12-leaf stage but before the 2 WAFB stage). Our data showed the opposite effect, with more <sup>14</sup>C-glyphosate absorption at the 12-leaf stage than at the eight-leaf stage in PDS applications, but similar levels of absorption when averaged over POST and PDS treatments. Differences in results from these two studies may be attributed to treatment at slightly different growth stages, the use of different glyphosate concentrations in herbicide applications, and the growing conditions for each study.

The influence of cotton growth stage on <sup>14</sup>C-glyphosate absorption may be a result of different developmental processes occurring at the different growth stages. For example, at the four-leaf growth stage, the cotton plant is undergoing primarily vegetative growth, whereas as the plant progresses to the 8-leaf, 12-leaf, and midbloom stage, it enters the initial reproductive phase by developing squares, blooms, and bolls (Mauney 1986). Environmental factors also may have affected <sup>14</sup>C-glyphosate absorption at each growth stage because applications to different stages of plants were not made on the same days.

These absorption data suggest a greater potential for glyphosate applied to either leaves or the stem to enter the plant when it is applied at reproductive stages as opposed to a vegetative stage. Absorption during the different reproductive stages (8-leaf, 12-leaf, and 2 WAFB) did not differ, suggesting that <sup>14</sup>C-glyphosate absorption reaches a plateau when cotton transfers from vegetative to reproductive stages.

### Treatment Placement and Growth Stage Effects on Translocation of <sup>14</sup>C-Glyphosate

Translocation of <sup>14</sup>C-glyphosate was dependent on growth stage at treatment and on the placement of the herbicide. Differences in <sup>14</sup>C-glyphosate translocation and accumulation between PDS and POST applications were evident. On a percentage of applied basis, the treatment placement main effect was significant for roots and fruiting branches, with greater <sup>14</sup>C-glyphosate translocation to roots of plants with PDS applications and to fruiting branches of plants receiving POST applications. On a concentration (Bq g<sup>-1</sup>) basis, the treatment placement main effect was significant for roots and stems, with greater translocation to both tissues in plants receiving PDS treatments than to those receiving POST treatments (Table 1). At all treatment timings, the concentration of <sup>14</sup>C-glyphosate in the roots was between 3.5 and 20 times greater in plants receiving PDS than POST treatments. This difference may be due to the proximity of the PDS herbicide treatment area to the roots, which may also serve as a strong metabolic sink (De Souza and Vieira da Silva 1987). There were no differences in the <sup>14</sup>C-glyphosate concentration in bolls with POST and PDS treatments, although there was translocation (between 0.9 and 1.9% of applied <sup>14</sup>C-glyphosate) to these tissues at the 2 WAFB stage (Table 2).

The general translocation patterns of <sup>14</sup>C-glyphosate applied to cotton plants POST or PDS differed. Plants receiving POST treatments at the four- and eight-leaf stages translocated <sup>14</sup>C-glyphosate primarily to the foliar plant portions, whereas plants that received PDS treatments at these stages translocated a considerable amount of <sup>14</sup>C-glyphosate to root and stem tissue as well as foliage (Tables 1 and 2).

TABLE 1. Distribution of <sup>14</sup>C-glyphosate in glyphosate-resistant Delta Pine 5415RR cotton (*Gossypium hirsutum*) treated at the 4-, 8-, or 12-leaf stage or at 2 wk after first bloom (WAFB) with either postemergence (POST) or POST-directed spray (PDS) treatments.<sup>a</sup>

Tissue <sup>b</sup>	<sup>14</sup> C-glyphosate concentration <sup>c</sup>												Inter-action <sup>d</sup>
	4-leaf			8-leaf			12-leaf			2 WAFB			
	POST	PDS	POST	PDS	POST	PDS	POST	PDS	POST	PDS	PDS		
Treated leaf or stem	2,802 (bc)	4,413 (a)	3,100 (ab)	1,385 (d)	1,960 (cd)	1,337 (c)	1,145 (d)	502 (e)				*	
Mature leaves	57 c(a)	153 b(a)	13 b(b)	30 c(b)	24 b(b)	15 b(b)	11 b(c)	8 cd(c)				NS	
Immature leaves and buds	256 a(a)	261 ab(a)	28 ab(c)	113 b(b)	19 b(cd)	15 b(cd)	66 a(cd)	19 bc(d)				*	
Stems	127 b(ab)	336 a(a)	21 b(d)	66 bc(c)	77 a(cd)	89 a(bc)	21 ab(e)	12 cd(e)				NS	
Roots	53 c(b)	1,029 a(a)	57 a(b)	837 a(a)	6 c(c)	126 a(b)	23 ab(c)	83 a(b)				NS	
Fruiting branches	N/A	N/A	N/A	N/A	56 bc(a)	13 b(a)	18 ab(ab)	6 d(b)				NS	
Squares	N/A	N/A	N/A	N/A	13 bc(a)	10 b(a)	48 ab(a)	46 b(a)				NS	
Bolls	N/A	N/A	N/A	N/A	N/A	N/A	23 ab(a)	11 bcd(a)				N/A	

<sup>a</sup> Reported means are actual means; however, all statistical analyses were performed on log-transformed means.

<sup>b</sup> Means in each row were separated using Fisher's protected LSD. Means followed by the same letter in parentheses are not significantly different at  $\alpha = 0.05$ .

<sup>c</sup> Means in each column, excluding treated leaf or stem, were separated using Fisher's protected LSD. Means followed by the same letter without parentheses are not significantly different at  $\alpha = 0.05$ .

<sup>d</sup> Treatment placement by growth stage at treatment interaction was significant at \*  $\alpha = 0.05$ ; NS, not significant.

TABLE 2. Distribution of  $^{14}\text{C}$ -glyphosate in glyphosate-resistant Delta Pine 5415RR cotton (*Gossypium hirsutum*) treated at the 4-, 8-, or 12-leaf stage or at 2 wk after first bloom (WAFB) with either postemergence (POST) or POST-directed spray (PDS) treatments.

Tissue <sup>a</sup>	$^{14}\text{C}$ -glyphosate absorption <sup>b,c</sup>								LSD <sup>a</sup>	Inter-action <sup>c</sup>
	4-leaf		8-leaf		12-leaf		2 WAFB			
	POST	PDS	POST	PDS	POST	PDS	POST	PDS		
	%									
Leaf wash	72.0	64.0	72.0	2.20	67.0	33.0	70.0	34.0	18.9	NS
Treated leaf or stem	10.0	12.2	26.8	11.7	26.7	31.2	13.2	19.0	11.3	*
Mature leaves	0.6 b	2.4 a	1.0 a	2.4 b	4.4 ab	3.3 bc	2.5 a	2.0 b	2.8	NS
Immature leaves and buds	0.9 a	0.7 a	0.6 a	3.2 b	0.5 b	0.5 c	2.3 a	0.6 b	2.1	*
Stems	0.6 b	0.7 a	0.7 a	1.6 b	11.2 ab	11.6 a	7.0 a	7.7 b	7.1	NS
Roots	0.1 c	3.2 a	1.0 a	13.9 a	0.4 b	8.2 ab	2.1 a	10.0 a	5.3	NS
Fruiting branches	N/A	N/A	N/A	N/A	6.4 ab	1.9 c	5.9 a	1.5 b	7.5	NS
Squares	N/A	N/A	N/A	N/A	0.2 b	0.3 c	0.8 a	3.7 b	0.9	NS
Bolls	N/A	N/A	N/A	N/A	N/A	N/A	1.9 a	0.9 b	1.6	N/A

<sup>a</sup> Means in each row were separated using Fisher's protected LSD at  $\alpha = 0.05$ .

<sup>b</sup> Means in each column, excluding the treated leaf or stem, were separated using Fisher's protected LSD. Means followed by the same letter are not significantly different at  $\alpha = 0.05$ .

<sup>c</sup> Treatment placement by growth stage at treatment interaction was significant at \*  $\alpha = 0.05$ ; NS, not significant.

Plants receiving POST applications at the 12-leaf growth stage translocated  $^{14}\text{C}$ -glyphosate primarily to the stem, fruiting branches, and leaves, whereas PDS plants translocated  $^{14}\text{C}$ -glyphosate primarily to stems and roots.

The concentration ( $\text{Bq g}^{-1}$ ) of  $^{14}\text{C}$ -glyphosate in all vegetative tissues (mature leaves, immature leaves and buds, roots, stems, and treated leaf or stem) decreased as the plant growth stage at treatment increased from the four-leaf stage to 2 WAFB (Table 1). This suggests that as plants grew, the concentration of  $^{14}\text{C}$ -glyphosate was diluted in vitro because of the greater mass of tissue at each application timing. The growth stage at treatment main effect was significant on a percentage of applied basis for mature leaves, roots, stems, treated leaf or stems, and squares. Squares, as soon as evident on the plant at the 12-leaf stage, began to accumulate  $^{14}\text{C}$ -glyphosate. The amount of  $^{14}\text{C}$ -glyphosate translocated to squares in PDS applications increased from 0.3 to 3.7% of applied  $^{14}\text{C}$ -glyphosate from the 12-leaf to 2 WAFB stage (Table 2). Because of their physiologically active growing/maturing state, they likely served as stronger metabolic sinks at the 2 WAFB than at 12-leaf stage, accounting for greater translocation of  $^{14}\text{C}$ -glyphosate. Significant interactions between the main effects of treatment placement and growth stage at treatment occurred on a concentration ( $\text{Bq g}^{-1}$ ) and percentage of applied basis for treated leaf or stems and immature leaves and buds.

At 2 WAFB applications,  $^{14}\text{C}$ -glyphosate distribution as percentage of applied becomes more homogeneous within all tissues of both POST- and PDS-treated plants than it was in earlier growth stages (Table 2). Wullschleger and Oosterhuis (1990) reported that reproductive-stage cotton plants cannot produce sufficient photosynthate to feed developing bolls by subtending leaves (leaves directly opposite of bolls) alone. Substantial translocation of photosynthate from adjacent leaves and leaves outside the main stem node is necessary, suggesting that the source-to-sink patterns of vegetative-state cotton differ vastly from those of reproductive cotton. Because glyphosate generally follows the pattern of photoassimilate in plants (Sandberg et al. 1980; Wyrill and Burnside 1976), translocation of  $^{14}\text{C}$ -glyphosate therefore likely would be affected in a developmental stage-de-

pendent manner. Photosynthate, and thus glyphosate during reproductive growth, may fail to accumulate in tissues that were sinks during vegetative stages and, instead, may begin to accumulate in different tissues during reproductive growth. Other studies monitoring the patterns of photosynthate transport in cotton using  $^{14}\text{C}$ -sucrose found that sucrose accumulated in roots during vegetative stages, but upon initiation of bolls, the roots did not continue as a major sink (Sabbe and Cathey 1969). Source-sink relations 2 WAFB are likely very different than at earlier growth stages, possibly accounting for the more homogeneous  $^{14}\text{C}$ -glyphosate translocation patterns.

### Distribution of $^{14}\text{C}$ -Glyphosate in Cotton Throughout its Life Cycle

The objective of this study was to measure the amount of  $^{14}\text{C}$ -glyphosate, applied POST at the four- and eight-leaf stages, remaining in plant tissues at various growth stages up to cutout (fewer than five nodes above the highest first position of white bloom). The amount of  $^{14}\text{C}$ -glyphosate recovered in plants treated at the four-leaf stage was less than for plants treated at the eight-leaf stage at each growth stage harvest (Table 3). This observation would suggest that degradation of nonabsorbed  $^{14}\text{C}$ -glyphosate occurs on the leaf surface or that absorbed  $^{14}\text{C}$ -glyphosate is exuded from roots or lost to the atmosphere to some extent. These processes are enhanced the longer the  $^{14}\text{C}$ -glyphosate label is left on the plant. McAllister and Haderlie (1985) reported recovery averaging 42% of applied  $^{14}\text{C}$ -glyphosate in an outdoor study in which the  $^{14}\text{C}$ -glyphosate label was left on treated Canada thistle [*Cirsium arvense* (L.) Scop.] plants for 8 d. They hypothesize that  $^{14}\text{C}$  loss over time could be from translocation of  $^{14}\text{C}$  beyond the region from which roots were collected, from possible metabolism of the herbicide (by the plant or by leaf surface microbiota) with a consequent loss of  $^{14}\text{CO}_2$ , or by rainfall wash-off, regardless of protective measures. The time-dependent loss of glyphosate from cotton plants mirrors the fate of glyphosate applied to crops under field conditions during a growing season (Rodrigues et al. 1982). Therefore, at the cutout stage in the

TABLE 3. Distribution of foliar-applied  $^{14}\text{C}$ -glyphosate in glyphosate-resistant Delta Pine 5415RR cotton (*Gossypium hirsutum*) treated at the 4- and 8-leaf stages and harvested at various growth stages.<sup>a</sup>

Tissue <sup>b</sup>	$^{14}\text{C}$ -glyphosate absorption (treatment stage by harvest stage) <sup>c</sup>										Inter-action <sup>d</sup>
	4-leaf					8-leaf					
	8-leaf	12-leaf	Midbloom	Cutout	%	8-leaf	12-leaf	Midbloom	Cutout	%	
Leaf wash	38.0 (ab)	12.8 (d)	16.7 (cd)	10.3 (d)	44.1 (a)	27.9 (bc)	24.4 (c)	27.2 (c)	27.2 (c)	27.2 (c)	NS
Treated leaf	41.4 (bcd)	31.8 (cd)	10.9 (d)	12 (d)	48.9 (ab)	42.4 (abc)	49.8 (a)	28.7 (abc)	28.7 (abc)	28.7 (abc)	*
Mature leaves	2.9 a(abcd)	7.8 a(a)	3.3 ab(ab)	5.7 a(ab)	2.1 a(bcd)	1.8 abc(d)	3.4 a(abcd)	2.0 a(cd)	2.0 a(cd)	2.0 a(cd)	NS
Immature leaves and buds	1.3 ab(a)	0.6 c(a)	1.1 bc(a)	0.4 d(a)	0.5 b(a)	0.9 bc(a)	0.5 cd(a)	0.7 ab(a)	0.7 ab(a)	0.7 ab(a)	NS
Roots	1.1 b(ab)	0.6 c(ab)	0.5 c(b)	2.2 b(a)	1.2 ab(ab)	0.4 c(b)	1.7 bc(a)	0.5 b(b)	0.5 b(b)	0.5 b(b)	*
Stems	1.5 ab(b)	4.3 ab(a)	1.6 ab(ab)	2.7 ab(ab)	1.7 a(ab)	1.9 ab(ab)	0.9 bcd(b)	1.8 a(ab)	1.8 a(ab)	1.8 a(ab)	NS
Fruiting branches	N/A	1.9 b(a)	1.4 bc(a)	4.7 ab(a)	2.2 a(a)	2.2 a(a)	1.8 ab(a)	1.1 ab(a)	1.1 ab(a)	1.1 ab(a)	NS
Squares	N/A	0.1 d(b)	0.2 d(b)	0.4 c(bc)	0.1 c(bc)	0.8 abc(a)	0.5 d(a)	0.1 c(c)	0.1 c(c)	0.1 c(c)	*
Bolls	N/A	N/A	0.5 cd(b)	1.7 b(a)	N/A	N/A	0.5 cd(ab)	2.5 a(a)	2.5 a(a)	2.5 a(a)	NS
Total recovery	86.2	60.0	36.2	40.1	99.5	78.3	83.5	64.6	64.6	64.6	

<sup>a</sup> Reported means are actual means; however, all statistical analyses were performed on the log-transformed means.

<sup>b</sup> Means in each row were separated using Fisher's protected LSD. Means followed by the same letter in parentheses are not significantly different at  $\alpha = 0.05$ .

<sup>c</sup> Means in each column, excluding the treated leaf, were separated using Fisher's protected LSD. Means followed by the same letter without parentheses are not significantly different at  $\alpha = 0.05$ .

<sup>d</sup> Treatment stage by harvest stage interaction was significant at \*  $\alpha = 0.05$ ; NS, not significant.

current study, up to 30% of glyphosate applied at the four-leaf stage and 39% of glyphosate applied at the eight-leaf stage remained within the cotton plant (Table 3).

Throughout the life cycle of cotton labeled with  $^{14}\text{C}$ -glyphosate, distribution patterns change. Translocation of  $^{14}\text{C}$ -glyphosate to root tissue was growth stage-dependent. In plants treated at the four-leaf stage, accumulation of  $^{14}\text{C}$ -glyphosate in root tissue starts out at  $96 \text{ Bq g}^{-1}$ , or 1.1% of applied  $^{14}\text{C}$ -glyphosate (Tables 3 and 4). As the plants entered reproductive stages, (12-leaf and midbloom), accumulation in the roots dropped significantly. Root growth in cotton increases as the plant develops until the onset of fruiting (McMichael 1980). Once fruiting commences, growth of aboveground tissues continue, but root growth is suppressed (Crowther 1934; McMichael 1980). It follows, therefore, that as fruiting is initiated, more photosynthate, and thus  $^{14}\text{C}$ -glyphosate, is diverted to developing fruits and less to roots. Our data show a reaccumulation of  $^{14}\text{C}$ -glyphosate in the roots at the cutout stage in plants treated at the four-leaf stage (Tables 3 and 4). This reaccumulation could again be a demonstration of source-sink patterns in the plant. As the plant is exiting the reproductive phase, developed bolls may cease to be a major sink. Because cotton is a perennial plant, it is possible that late in the season, the plant begins transporting resources to the belowground portion of the plant in preparation for perennial regrowth the following season.

At all cotton growth stages, accumulation based on percentage of applied  $^{14}\text{C}$ -glyphosate, when applied at the four- and eight-leaf stages, was highest in mature leaf tissue (Table 3). Mature leaves constituted  $> 50\%$  of the entire dry weight biomass of the plant—considerably more than any other organ (data not shown). Because  $^{14}\text{C}$ -glyphosate accumulation ( $\text{Bq g}^{-1}$ ) in mature leaves is similar to other tissues (Table 4), it does not appear to be an area of high  $^{14}\text{C}$ -glyphosate accumulation. Mature stem and subtending leaves are the major sources of assimilates for developing bolls (Benedict and Kohel 1975) and would thus not be expected to accumulate  $^{14}\text{C}$ -glyphosate as sink tissues do.

As cotton progresses through its growth stages, the percentage of applied  $^{14}\text{C}$ -glyphosate increases in reproductive tissues. In plants treated at the four-leaf stage, the percentage of applied  $^{14}\text{C}$ -glyphosate accumulating in the reproductive areas (sum of percentages in fruiting branches, squares, and bolls) increases from 2% at the 12-leaf stage to 6.8% at cutout. In plants treated at the eight-leaf stage, accumulation increases from 3.0% at the 12-leaf stage to 3.7% at cutout, with 2.5% of the applied  $^{14}\text{C}$ -glyphosate accumulating in the bolls (Table 3).

The concentration of  $^{14}\text{C}$ -glyphosate ( $\text{Bq g}^{-1}$ ) also increased in the reproductive tissues. At all growth stages following  $^{14}\text{C}$ -glyphosate treatment at the eight-leaf stage, the concentration of  $^{14}\text{C}$ -glyphosate in the squares is equivalent to or higher than the concentration in any other tissue (Table 4). The concentration reaches a maximum at the 12-leaf stage, with  $43 \text{ Bq }^{14}\text{C}$ -glyphosate  $\text{g}^{-1}$  square tissue, and at the cutout stage for bolls, with  $12 \text{ Bq }^{14}\text{C}$ -glyphosate  $\text{g}^{-1}$  boll tissue. The rate of  $^{14}\text{C}$ -glyphosate applied to the  $5\text{-cm}^2$  area was equal to 17% of the recommended field application rate of  $1.12 \text{ kg ai ha}^{-1}$ . Thus in a field application, glyphosate accumulation theoretically would be 5.9 times greater for every  $5\text{-cm}^2$  area treated than in this study. Ac-

TABLE 4. Distribution of foliar-applied  $^{14}\text{C}$ -glyphosate in glyphosate-resistant Delta Pine 5415RR cotton (*Gossypium hirsutum*) treated at the 4- and 8-leaf stages and harvested at various growth stages.<sup>a</sup>

Tissue <sup>b</sup>	$^{14}\text{C}$ -glyphosate concentration (treatment stage by harvest stage) <sup>c</sup>										Inter-action <sup>d</sup>		
	4-leaf					8-leaf							
	8-leaf	12-leaf	Midbloom	Cutout	10-leaf	12-leaf	Midbloom	Cutout	8-leaf	12-leaf			
	Bq g <sup>-1</sup> dry weight												
Treated leaf	2,884 (a)	1,560 (abc)	612 (cd)	579 (d)	2,376 (ab)	1,886 (ab)	2,269 (ab)	1,757 (abc)	2,269 (ab)	1,886 (ab)	2,269 (ab)	1,757 (abc)	*
Mature leaves	31 a(a)	30 a(a)	12 ab(abc)	20 a(ab)	18 bc(abc)	12 b(c)	17 ab(abc)	9 ab(bc)	17 ab(abc)	12 b(c)	17 ab(abc)	9 ab(bc)	NS
Immature leaves and buds	64 a(a)	21 ab(b)	19 a(b)	8 a(b)	30 abc(ab)	23 ab(b)	8 bc(b)	21 a(b)	8 bc(b)	23 ab(b)	8 bc(b)	21 a(b)	NS
Roots	96 a(a)	8 bc(c)	5 c(c)	17 a(bc)	35 ab(ab)	8 b(c)	21 ab(bc)	16 ab(c)	21 ab(bc)	8 b(c)	21 ab(bc)	16 ab(c)	NS
Stems	39 a(a)	19 ab(bc)	6 bc(d)	8 a(cd)	23 abc(ab)	11 b(cd)	4 c(d)	7 ab(d)	4 c(d)	11 b(cd)	4 c(d)	7 ab(d)	NS
Fruiting branches	N/A	11 abc(a)	7 c(a)	13 a(a)	10 c(a)	14 b(a)	6 bc(a)	4 b(a)	6 bc(a)	14 b(a)	6 bc(a)	4 b(a)	NS
Squares	N/A	7 bc(bc)	9 bc(c)	13 a(c)	32 a(a)	43 a(a)	37 a(ab)	10 ab(c)	37 a(ab)	43 a(a)	37 a(ab)	10 ab(c)	*
Bolls	N/A	N/A	6 c(a)	8 a(a)	N/A	N/A	9 b(a)	12 ab(a)	9 b(a)	N/A	9 b(a)	12 ab(a)	NS

<sup>a</sup> All means underwent logarithmic transformation. Reported means are actual means; however, all statistical analyses were performed on log-transformed means.

<sup>b</sup> Means in each row were separated using Fisher's protected LSD. Means followed by the same letter in parentheses are not significantly different at  $\alpha = 0.05$ .

<sup>c</sup> Means in each column, excluding the treated leaf, were separated using Fisher's protected LSD. Means followed by the same letter without parentheses are not significantly different at  $\alpha = 0.05$ .

<sup>d</sup> Treatment stage by harvest stage interaction was significant at \*  $\alpha = 0.05$ ; NS, not significant.

accumulation of  $^{14}\text{C}$ -glyphosate in squares from cotton at the 12-leaf stage could reach 253.7 Bq or 0.48  $\mu\text{g}$  glyphosate  $\text{g}^{-1}$  of tissue dry weight per 5- $\text{cm}^2$  area treated. Bolls from cotton at cutout could reach 0.14  $\mu\text{g}$  glyphosate  $\text{g}^{-1}$  of tissue dry weight per 5- $\text{cm}^2$  area treated. The biological significance of this level of accumulation in reproductive tissue needs to be determined.

These data not only indicate that  $^{14}\text{C}$ -glyphosate remains in the plant tissue throughout the growing season but that  $^{14}\text{C}$ -glyphosate accumulates in reproductive tissues. Because glyphosate metabolism by plants is negligible (Duke 1988), the  $^{14}\text{C}$ -glyphosate remaining in the plant is assumed to be nondegraded glyphosate. If these reproductive tissues are not as resistant to glyphosate as other tissue types because of differential expression of the CP4-EPSPS gene, the accumulation of glyphosate could potentially lead to tolerance problems in the reproductive tissues.

Overall, our results show that absorption and translocation differ with PDS and POST applications of  $^{14}\text{C}$ -glyphosate.  $^{14}\text{C}$ -glyphosate absorption on an equal surface area with stem applications was greater than with foliar treatments. However, the practical implications of this observation are unknown. Because leaf tissue constitutes a greater total surface area than stem surface area on cotton plants, total foliar absorption likely is still greater than stem absorption in field applications.

Translocation of  $^{14}\text{C}$ -glyphosate in plants with foliar applications was primarily to foliar plant tissue, whereas with stem applications, translocation to roots and stem tissue was greatest. Thirty to 37% of the  $^{14}\text{C}$ -glyphosate applied to plants at the four- and eight-leaf stages remains within plants at cutout.  $^{14}\text{C}$ -glyphosate accumulates in reproductive tissues such as squares and bolls beginning at the 10-leaf stage and increases in later growth stages, especially in plants treated at the eight-leaf stage. In general, these data may offer some explanation for the observations of greater reproductive structure loss in glyphosate-resistant cotton fields in the southeastern United States. However, further research investigating the expression of the CP4-EPSPS gene in reproductive tissues, the biological significance of glyphosate accumulation in tissues, and environmental effects on glyphosate-resistant cotton growth and tolerance are needed.

## Sources of Materials

<sup>1</sup> Metro-Mix 360. Scotts-Sierra Horticultural Products Co., 14111 Scottslawn Road, Marysville, OH 43041.

<sup>2</sup> Sigma Co., 11542 Fort Mims Drive, St. Louis, MO 63146-3510.

<sup>3</sup> Induce® nonionic low-foam wetter/spreader adjuvant contains 90% nonionic surfactant (alkylaryl polyoxyalkane ether and isopropanol), free fatty acids, and 10% water. Helena Chemical Co., Suite 500, 6075 Poplar Avenue, Memphis, TN 38137.

<sup>4</sup> Coffee mill. Mr. Coffee, 24700 Miles Road, Bedford Heights, OH 44146-1399.

<sup>5</sup> Harvey biological oxidizer. J. Harvey Instrument Corporation, 123 Patterson Street, Hillsdale, NJ 07642.

<sup>6</sup> Statistical Analysis Systems (SAS) software. Ver. 8. SAS Institute, Inc., Box 8000, SAS Circle, Cary, NC 27513.

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