ABSTRACT

THOMAS, WALTER EDWARD. Physiological evaluations of translocation of glyphosate in glyphosate resistant crops including interaction with cotton growth regulators. (Under the direction of Dr. John W. Wilcut.)

Field studies were conducted in 2004 to evaluate corn tolerance, weed control, grain yield, and net returns in transgenic and non-transgenic corn with various herbicide systems. No significant differences between hybrid systems were observed for weed control. Grain yield was variable between hybrids and locations due to environmental differences. Consequently, net returns for each hybrid system within a location were also variable.

Studies were conducted at three locations in North Carolina in 2004 to evaluate density-dependent effects of glyphosate-resistant (GR) corn on GR cotton growth and lint yield. The examined GR corn densities had a significant effect on cotton yield, but not as significant as many other problematic grass and broadleaf weeds.

Two studies were conducted to investigate the influence of corn growth stage on the absorption and translocation of glyphosate in glyphosate-resistant (GR) corn. Regardless of corn growth stage, the leaves above the treated leaf and roots were the greatest sinks for ^{14}C-glyphosate. These data suggest that reproductive tissues such as the tassel and ear shoots can accumulate ^{14}C-glyphosate at higher concentrations that other tissues, especially when the herbicide treatment is applied postemergence after the V6 stage.

Studies were conducted to evaluate absorption and translocation of ^{14}C-glyphosate in both commercial glyphosate-resistant (GR) cotton events [GR event 1, released 1997 (GRE1) and GR event 2, released 2006 (GRE2)] were evaluated at the 4-leaf (L) and 8-L growth stages. Glyphosate absorption, as a percentage of applied, increased over time. In

8-L cotton, glyphosate absorption was not different between events. Glyphosate translocation patterns were not different between events or harvest timings and exhibited a source-sink relationship. Based on the percentage of ^{14}C exported out of the treated leaf, glyphosate and sucrose translocation patterns were similar, indicating that glyphosate may be used as a photoassimilate model in GRE2 cotton.

Studies examined various morphological characteristics and ^{14}C-glyphosate translocation in cotton as influenced by ^{14}C-treatment timing and mepiquat chloride (MC). No significant differences in plant height, leaf area, and specific leaf weight were observed for any treatment. Dry weight of first position fruits on nodes 1, 2, and 3 of MC treated plants accumulated greater biomass compared to fruits on non-treated plants. No significant observations were found for ^{14}C-glyphosate translocation. These data support previous research that showed increased fruit weight and provides insight into the potential for MC treatment to alter source to sink relationship in cotton.

Studies examined various morphological characteristics and ^{14}C-glyphosate translocation in cotton as influenced by cotton plant growth regulator regimes and source leaf. MC and MP reduced cotton height and the number of nodes per plant. Total fruit retention and first position fruit retention were not influenced by any treatment. Based on these data, MC and MP do not influence ^{14}C-glyphosate translocation.

Greenhouse studies were conducted to evaluate the rain-free requirement for mepiquat chloride and mepiquat chloride plus cyclanilide with and without surfactant and to evaluate absorption and translocation of cyclanilide. Based on these data, a rain-free period of 8 hours is needed to maximize efficacy, regardless of plant growth regulator or the use of surfactant. Absorption of cyclanilide ranged from 11 to 15% at 3 and 48 HAT,

respectively. Averaged over harvest intervals, 18% of the applied cyclanilide remained in the treated leaf while 1.7 and 6.5% of the applied cyclanilide was found in the above and below treated leaf tissue, respectively.

PHYSIOLOGICAL EVALUATIONS OF TRANSLOCATION OF GLYPHOSATE IN GLYPHOSATE RESISTANT CROPS INCLUDING INTERACTION WITH COTTON GROWTH REGULATORS

By

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BIOGRAPHY

Walter E. Thomas was born in Sanford, North Carolina on June 12, 1979 to Neill B. Thomas, Jr. and Frances G. Thomas. He was raised in Lillington, North Carolina, a small rural town. He graduated in 1997 from Western Harnett High School as a North Carolina Scholar and Honor Student. Following graduation, he enrolled at North Carolina State University where he earned a bachelor of science degree in Agronomy with a concentration in Turfgrass Management. In October 2000, he earned the American FFA degree, the highest degree bestowed upon FFA members. He graduated sum cum laude, a College of Agriculture and Life Sciences Honor Student, and a University Scholar. Walter earned a Master of Science in Crop Science and a minor in Entomology from North Carolina State University in December of 2003. Following graduation, he pursued a doctor of philosophy in Crop Science at North Carolina State University.

While pursuing his degrees, Walter has won awards in the graduate student poster contests at the Southern Weed Science Society and Weed Science Society of North Carolina and graduate student oral presentation contests at the Beltwide Cotton Conference. He was also awarded the Deere & Co. Graduate Fellowship from the American Society of Agronomy. He was a participant and award winner in weed contests conducted by the Northeastern and Southern Weed Science Societies. In 2005, he was a member of the first place team at the Northeastern Weed Science Society Weed Contest in Lancaster, PA.

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TABLE OF CONTENTS

LIST OF TABLES

GLYPHOSATE-RESISTANT CORN INTERFERENCE IN GLYPHOSATE-RESISTANT COTTON

INFLUENCE OF CORN GROWTH STAGE ON ABSORPTION AND TRANSLOCATION OF GLYPHOSATE IN GLYPHOSATE-RESISTANT CORN

ABSORPTION AND TRANSLOCATION OF GLYPHOSATE AND SUCROSE IN GLYPHOSATE-RESISTANT COTTON

TRANSLOCATION OF SPOT APPLICATIONS OF GLYPHOSATE AS INFLUENCED BY MEPIQUAT CHLORIDE APPLICATIONS IN COTTON

TRANSLOCATION OF ^{14}C-GLYPHOSATE AS INFLUENCED BY VARIOUS PLANT GROWTH REGULATORS IN COTTON

RAIN-FREE REQUIREMENT AND PHYSIOLOGICAL PROPERTIES OF COTTON PLANT GROWTH REGULATORS

LIST OF FIGURES

GLYPHOSATE-RESISTANT CORN INTERFERENCE IN GLYPHOSATE-RESISTANT COTTON

ABSORPTION AND TRANSLOCATION OF GLYPHOSATE AND SUCROSE IN GLYPHOSATE-RESISTANT COTTON

RAIN-FREE REQUIREMENT AND PHYSIOLOGICAL PROPERTIES OF COTTON PLANT GROWTH REGULATORS

INTRODUCTION

Objective 1. Herbicide-resistant (HR) crops are becoming increasingly prevalent in the southeastern United States. Herbicide-resistant corn hectarage has steadily increased from 7% in 2000 to 26% in 2005 (USDA-NASS 2000, 2005). The use of HR cotton has been more widespread with 46 and 61% planted in 2000 and 2005, respectively (USDA-NASS 2000, 2005). In North Carolina, 78% of the planted cotton in 2005 contained a herbicide-resistant trait (USDA-NASS 2005). In addition to the wide-spread use of glyphosate-resistant (GR) corn and cotton, many of the weed management systems within each cropping system utilize only glyphosate. Additional herbicidal options like metolachlor and pendimethalin included in some cotton weed management systems are also registered in corn and are unlikely to control volunteer corn. The combination of continuous use of GR cropping systems with the inclusion of only glyphosate allows for growth and subsequent competition of GR volunteers (York et al. 2004, 2005).

Since the registration of GR cotton, less than 50% of the NC hectarage receives any residual preemergence treatment (A. C. York, personal communication). A vast majority of this hectarage is only treated with glyphosate or glyphosate plus *S*-metolachlor for the first 4 to 5 wks after crop emergence. In addition, weather conditions like hurricanes commonly destroy corn. Therefore, GR corn volunteers are often left uncontrolled. Since interference between GR corn and GR cotton has not been investigated, studies were conducted to determine effects of a range of GR corn densities on GR cotton growth and yield and to evaluate growth of GR corn as affected by plant density (Chapter 1, pp. 17 - 39).

GR corn was taller than GR cotton as early as 25 d after planting, depending on location. A GR corn density of 5.25 plant/m of crop row reduced late-season cotton height by 49, 24, and 28% at Clayton, Lewiston-Woodville, and Rocky Mount, respectively, compared to weed-free cotton height. Using the rectangular hyperbola model with the asymptote (a) constrained to 100% maximum yield loss, the estimated coefficient *i* (yield loss per unit density as density approaches zero) was 9, 5, and 5 at Clayton, Lewiston-Woodville, and Rocky Mount, respectively. The examined GR corn densities had a significant effect on cotton yield, but not as significant as many other problematic grass and broadleaf weeds.

Objective 2. Glyphosate has been shown to negatively influence various reproductive characteristics in GR corn and cotton (Pline et al. 2002a; Thomas et al. 2004). Investigations concluded that yield reductions in GR cotton were due to lower CP4-5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) expression in male reproductive portions of flowers compared to vegetative tissues (Pline et al. 2002a). In addition to lower EPSPS levels, pollen viability was reduced and stigma length was increased following glyphosate treatments (Pline et al. 2002a, 2002b, 2002c; Yasour et al. 2000). In GR corn, pollen viability reductions were evident with glyphosate treatments after the V6 stage, however, no yield reductions were observed with any glyphosate treatment combination (Thomas et al. 2004). The combination of tassel initiation [as early as two wks after corn emergence (Kiesselbach 1992)] and the source-to-sink translocation of glyphosate (Gougler and Geiger 1981; Hetherington et al. 1999; McAllister and Haderlie 1985; Pline et al. 2001; Sandberg et al. 1980; Wyrill and Burnside 1976; Viator et al. 2003) may partially explain the observed pollen viability reductions (Thomas et al. 2004). Based

on this hypothesis, our objectives were to evaluate absorption and translocation of glyphosate applied at the V4, V6, or V8 stage using various harvest timings. The first study evaluated the immediate effects (3 and 7 DAT) on absorption and translocation of glyphosate. A second study evaluated the distribution of foliar-applied glyphosate (V4, V6, and V8) by harvesting at four vegetative and reproductive stages (V8, V12, V16, and R1) (Chapter 2, pp. 40 - 62).

In the second study, 42 to 60% of the applied ^{14}C-glyphosate remained in the corn tissues at anthesis. The leaves above the treated leaf and roots accumulated the greatest amounts of ^{14}C-glyphosate, regardless of corn growth stages. When plants were treated at V4, V6, and V8 stages, the concentration of ^{14}C-glyphosate in the tassel at the V12 harvest timing was 184, 431, and 921 Bq g^{1} dry tissue, respectively. Likewise, increasing levels of ^{14}C-glyphosate concentrations between corn growth stages were also observed in ear shoots. These data suggest that reproductive tissues such as the tassel and ear shoots can accumulate ^{14}C-glyphosate at higher concentrations than other tissues, especially when the herbicide is applied POST after the V6 stage.

Objective 3. In addition to laboratory analyses of CP4-EPSPS expression levels (Pline et al. 2002a), determination of shikimic acid levels (which accumulates following inhibition of EPSPS) (Pline et al. 2002b; Viator et al. 2003), and floral morphology assessments (Pline et al. 2002b), several researchers have reported abscission of first position bolls (Jones and Snipes 1999; Viator et al. 2003, 2004), which in some years resulted in delayed maturity. Depending on year, Viator et al. (2004) reported an 8% yield reduction following applications of glyphosate POST at 0.84 kg ae/ha followed by glyphosate postemergence-directed at 0.84 kg/ha compared to treatments that did not include

glyphosate. The lack of glyphosate tolerance in reproductive tissues of glyphosate-resistant event 1 (GRE1) lead to the development of a new generation of GR cotton.

The new generation, glyphosate-resistant event 2 (GRE2) cotton, uses an identical resistance gene but different promoters as compared to the original resistance technology (Anonymous 2005). The use of alternate promoters has increased the tolerance in reproductive portions of the plant while maintaining tolerance levels in vegetative parts (Anonymous 2005). Consequently, glyphosate can be applied POST up to 7 days before harvest. Multiple researchers have investigated the physiological behavior of ^{14}C-glyphosate in GRE1 cotton (Pline et al. 2001, 2002a; Viator et al. 2003), however limited research investigating similar responses to glyphosate in GRE2 cotton has been reported. Thus, our objective was to evaluate absorption and translocation of glyphosate in these two events at different cotton growth stages (Chapter 3, pp. 63 - 85).

Glyphosate absorption, as a percentage of applied, increased over time with 29 and 36% absorption at 7 DAT in GRE1 and GRE2 cotton at the 4-lf growth stage, respectively. In 8-lf cotton, glyphosate absorption (33% at 7 DAT) was not different between events. Glyphosate translocation patterns were not different between events or harvest timings and exhibited a source-to-sink relationship. Observed translocation differences between cotton growth stages were probably due to reduced glyphosate export from the treated leaf of 8-lf cotton.

Objective 4. Previous photoassimilate movement research in cotton used radiolabeled substrates including CO_2 and sucrose (Ashley 1972; Benedict and Kohel 1975; Benedict et al. 1973; Horrocks et al. 1978). However, these substrates are metabolized into multiple plant products, which may complicate analysis and data interpretation. Glyphosate, which

is not readily metabolized in plants (Duke 1988; Sandberg et al. 1980) and shares similar translocation patterns to sucrose (Dewey and Appleby 1983; Tardif and Leroux 1993), may offer an additional tool in photoassimilate research. Glyphosate-resistant event 2 cotton in combination with glyphosate may provide tools to study photoassimilate translocation in cotton in the absence of glyphosate toxicity (Feng and Chiu 2005). Even though several researchers observed similar glyphosate and sucrose translocation patterns in a number of weed species (Dewey and Appleby 1983; Shieh et al. 1993; Tardif and Leroux 1993), a comparison of glyphosate and sucrose translocation in GR cotton or other GR crops has not been conducted. Thus, our objective was to compare glyphosate and sucrose translocation patterns in GRE2 cotton as influenced by cotton growth stage (Chapter 3, pp. 63 - 85).

Averaged over trials, ^{14}C compounds, and growth stages, cotton absorbed 28% of the applied dose at 14 d after treatment. Based on the percentage of ^{14}C exported out of the treated leaf, glyphosate and sucrose translocation patterns were similar, indicating that glyphosate may be used as a photoassimilate model in GRE2 cotton.

Objectives 5 and 6. Development and retention of cotton fruits are influenced by supply and demand of plant photoassimilates. The complex balance of these source-to-sink relationships varies by plant position and age (Ashley 1972; Benedict and Kohel 1975) as well as environmental conditions (Guinn 1982). Multiple researchers have investigated photoassimilate patterns in cotton with $^{14}CO_2$ (Ashley1972; Benedict and Kohel 1975; Horrocks et al. 1978) and ^{14}C-glyphosate (Feng and Chiu 2005). Ashley (1972) and Horrocks et al. (1978) showed that the subtending leaf of a fruit was the primary photoassimilate source.

Glyphosate, a commonly used systemic herbicide, is symplastic in nature and is translocated in the phloem following a source-to-sink relationship (Dewey and Appleby 1983; Sandberg et al. 1980; Tardif and Leroux 1993). Due to these properties, similar patterns of glyphosate and sucrose translocation have been reported in Canada thistle [*Cirsium arvense* (L.) Scop.], cotton, quackgrass [*Elyrigia repens* (L.) Nevski], and tall morningglory [*Ipomoea purpurea* (L.) Roth.] (Dewey and Appleby 1983; Harker and Dekker 1988; Klevorn and Wyse 1984; McAllister and Haderlie 1985; Shieh et al. 1993; Tardif and Leroux 1993; Thomas et al. 2006).

Many cotton production systems include the use of plant growth regulators (PGRs) to manage vegetative cotton growth. Mepiquat chloride and mepiquat pentaborate, two onium-type growth regulators commonly used in these systems, inhibit gibberillic acid synthesis by stopping the conversion of geranylgeranyl diphosphate to *ent*-kaurene, consequently reducing cell enlargement and the rate of cell division (Rademacher 2000; Srivastava 1993). The visual effects of these PGRs include reduced stem and leaf expansion and cotton reaching maturity earlier than cotton not treated with PGRs (Reddy et al. 1990, 1996; York 1983a, 1983b). However, cotton yield responses were variable (Kerby 1985). Since PGRs have been shown to alter cotton canopy architecture and fruit maturity, photoassimilate translocation patterns are presumably altered by these applications. Even though research has not confirmed differences in photoassimilate translocation in response to MC treatment using $^{14}CO_2$ technology (Zhao and Oosterhuis 2000), glyphosate and glyphosate-resistant technology offer new tools for investigating photoassimilate translocation patterns in cotton (Feng and Chiu 2005).

Since glyphosate movement has been correlated with sucrose movement in cotton (Thomas et al. 2006) and multiple weed species (Dewey and Appleby 1983; Harker and Dekker 1988; Klevorn and Wyse 1984; McAllister and Haderlie 1985; Shieh et al. 1993; Tardif and Leroux 1993), our objectives were to evaluate translocation of ^{14}C-glyphosate at different timings relative to a single MC treatment (Chapter 4, pp. 86 - 118) and to evaluate translocation of ^{14}C-glyphosate from various source leaves as influenced by multiple cotton PGR regimes (Chapter 5, pp. 119 - 143).

Due to the large number of plant parts, data for a five-node section of each plant was recorded for each parameter (the mainstem leaf on node B received the ^{14}C-glyphosate treatment). No significant differences in plant height, leaf area, and specific leaf weight were observed for any ^{14}C-glyphosate timing or MC combination. At the time of MC treatment and 21 DMC, total fruit retention was 85 and 70%, respectively. Even though all plants retained at least 86% of all first position fruits, a significant decline was observed from the day of MC treatment to 21 DMC (92 and 86%, respectively). Fresh and dry weight data showed similar responses with multiple first position fruits of MC-treated plants accumulating more biomass compared to first position fruits of non-treated plants. For dry weight of plants parts, first position fruits on nodes A, B, and C of MC-treated plants accumulated 30, 35, and 45% greater biomass, respectively, compared to first position fruits of non-treated plants. Absorption of ^{14}C-glyphosate was not influenced by either ^{14}C-glyphosate timing or MC treatment. Even though no significant observations were found on node A, several numerical observations offer support for reduced leaf expansion and increased biomass accumulation in response to MC treatment. In node A, second position fruits on MC-treated plants contained 68% more ^{14}C-glyphosate g^{-1} of dry

tissue than second position fruits of non-treated plants. In addition, numerical increases in ^{14}C-glyphosate concentration in the leaf and stem of the second position of node A in MC-treated plants were observed compared to the same parts of non-treated plants. On node D, first position fruits of MC-treated plant contained nearly 6 and 7 times the ^{14}C-glyphosate concentration at 0 and 21 DMC treatment compared to the respective timing for non-treated plants. In addition, all other comparisons with first position fruit were numerically greater with MC treatment. These data support previous research that showed increased fruit weight and provides insight into the potential for MC treatment to alter the source-to-sink relationship in reproductive cotton.

Studies examined various morphological characteristics and ^{14}C-glyphosate translocation in cotton as influenced by cotton plant growth regulator regimes [none, mepiquat chloride (MC), mepiquat pentaborate (MP)] and source leaf (^{14}C-glyphosate was applied to mainstem and sympodial leaves). Mepiquat chloride and MP reduced cotton height by 13 and 20%, respectively, compared to non-treated plants. Mepiquat chloride and MP reduced the number of mainstem nodes by 1.4 and 1.9 nodes plant^{-1}, respectively, compared to non-treated plants. Total fruit retention, first position fruit retention, low sympodial fruit retention (nodes 6-10), high sympodial fruit retention (nodes 11-15), and the location of the first sympodial branch were not influenced by either ^{14}C-glyphosate treatment or cotton PGR regime. On node 19, MC and MP reduced the leaf area of the mainstem leaf by 59 and 73%, compared to the mainstem leaf of non-treated plants. This observed reduction may be partially influenced by the reduction in cotton height and the number of nodes. Leaf area at other positions was only influenced by node and position within nodes. Absorption of ^{14}C-glyphosate was not influenced by either ^{14}C-glyphosate

placement or cotton PGR regime. Based on these data, MC and MP do not influence ^{14}C-glyphosate translocation, which presumably indicates no direct influence on photoassimilate translocation.

Objective 7. In 2006, a prepackaged mixture of mepiquat chloride and cyclanilide, which inhibits gibberellic acid synthesis and auxin transport, respectively, was registered for use in cotton. Compared to known polar auxin transport inhibitors (1-*N*-naphthylphthalamic acid and 2, 3, 4-triiodobenzoic acid) [12], Pederson [13] reported similar levels of inhibition of auxin transport with 10 µm of cyclanilide in etiolated coleoptiles of corn (*Zea mays*). Due to limited basipetal transport of auxin in cyclanilide treated plants, apical dominance is not maintained, allowing for increased lateral shoot growth [13-17].

Since the prepackaged mixture of mepiquat chloride plus cyclanilide has recently been registered, limited data are available with regard to rain-free requirements and distribution within cotton. Therefore, our objectives were (1) to determine the rain-free interval for mepiquat chloride and mepiquat chloride plus cyclanilide alone or in combination with a non-ionic surfactant and (2) to evaluate absorption and translocation of cyclanilide in cotton (Chapter 6, pp. 144 - 162).

No significant differences in the number of nodes, leaf area, and plant part fresh and dry weight were observed with any PGR treatment and rainfall simulation combination. Both plant growth regulators responded similarly to rainfall interval. As rain-free period increased, cotton height was reduced. Based on these data, a rain-free period of 8 hours is needed to maximize efficacy, regardless of the use of surfactant. Absorption of cyclanilide ranged from 11 to 15% at 3 and 48 HAT, respectively. Averaged over harvest intervals,

18% of the applied cyclanilide remained in the treated leaf while 1.7 and 6.5% of the applied cyclanilide was found in the above and below treated leaf tissue, respectively.

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Glyphosate-Resistant Corn Interference in Glyphosate-Resistant Cotton[1]

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Abstract: Studies were conducted at three locations in North Carolina in 2004 to evaluate density-dependent effects of glyphosate-resistant (GR) corn on GR cotton growth and lint yield. GR corn was taller than GR cotton as early as 25 d after planting, depending on location. A GR corn density of 5.25 plant/m of crop row reduced late-season cotton height by 49, 24, and 28% at Clayton, Lewiston-Woodville, and Rocky Mount, respectively, compared to weed-free cotton height. At Clayton, GR corn dry biomass per m crop row and GR corn seed biomass per m of crop row decreased linearly. The relationship between GR corn and GR cotton yield loss was described by the rectangular hyperbola model with the asymptote (*a*) constrained to 100% maximum yield loss. The estimated coefficient *i* (yield loss per unit density as density approaches zero) was 9, 5, and 5 at Clayton, Lewiston-Woodville, and Rocky Mount, respectively. The examined GR corn densities had a significant effect on cotton yield, but not as significant as many other problematic grass and broadleaf weeds.

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Nomenclature: Glyphosate, corn, *Zea mays* L., ZEAMX, 'DKC 69-71RR; cotton, *Gossypium hirsutum* L., 'FM 989RR', ST 4892RR'.

Keywords: Competition, economic threshold, models, weed biomass, weed density, plant height.

INTRODUCTION

Herbicide-resistant crops are becoming increasingly prevalent in the southeastern United States. Herbicide-resistant corn hectarage has steadily increased from 7% in 2000 to 26% in 2005 (USDA-NASS 2000, 2005). The use of herbicide-resistant cotton has been more widespread with 46 and 61% planted in 2000 and 2005, respectively (USDA-NASS 2000, 2005). In North Carolina, 78% of the planted cotton in 2005 contained a herbicide-resistant trait (USDA-NASS 2005). In addition to the wide-spread use of glyphosate-resistant (GR) corn and cotton, many of the weed management systems within each cropping system utilize only glyphosate. Additional herbicidal options like metolachlor and pendimethalin included in some cotton weed management systems are also registered in corn and are unlikely to control volunteer corn. Many graminicides are registered for use in cotton to control annual and perennial grasses and may be needed to control volunteer GR corn. The combination of continuous use of GR cropping systems with the inclusion of only glyphosate allows for growth and subsequent competition of GR volunteers (York et al. 2004, 2005).

Since the registration of GR cotton, less than 50% of the North Carolina hectarage receives any residual preemergence treatment (A. C. York, personal communication). A

vast majority of this hectarage is only treated with glyphosate or glyphosate plus *S*-metolachlor for the first 4 to 5 wks after crop emergence. In addition, weather conditions like hurricanes commonly destroy corn. Therefore, GR corn volunteers are often left uncontrolled. If an economic threshold is to be realized, data on weed interference must be collected for yield-loss prediction models (Coble and Byrd 1992). Since interference between GR corn and GR cotton has not been investigated, studies were conducted to determine effects of a range of GR corn densities on GR cotton growth and yield and to evaluate growth of GR corn as affected by plant density.

MATERIALS AND METHODS

Field experiments were conducted at the Central Crops Research Station near Clayton, NC, the Upper Coastal Plain Research Station near Rocky Mount, NC, and the Peanut Belt Research Station near Lewiston-Woodville, NC in 2004. Soil at these locations was a Norfolk loamy sand (fine-loamy, siliceous, thermic Typic Paleudults) having 0.9 to 1.1% organic matter and pH 5.8 to 6.0. All sites were disked and smoothed with a field cultivator, and pendimethalin at 0.84 kg ai/ha was applied preemergence (PRE). Cotton cultivars were 'FM 989RR' at Clayton and Lewiston-Woodville and 'ST 4892RR' at Rocky Mount. Seed were planted on conventional seedbeds at 15 seeds per m of cotton row on May 6, May 13, and May 11 at Clayton, Lewiston-Woodville, and Rocky Mount, respectively. Plots were 6 m long and four 91-cm rows at Lewiston-Woodville and Rocky Mount with four 97-cm rows at Clayton. Fertilization and pest management practices were standard for cotton production in North Carolina. Glyphosate at 0.84 kg ae/ha was applied as recommended by the registration (Anonymous 2005) up to eight-leaf cotton to control

emerged weeds. After the four-leaf stage, glyphosate was applied postemergence-directed to limit contact with cotton foliage. After the eight-leaf stage, weeds were removed by hand. The experimental design was a randomized complete block with treatments replicated three times.

On the day of cotton planting at each location, GR corn 'DKC 69-71RR' was planted at desired densities 15 cm from the crop row and evenly spaced along crop rows. Corn densities were 0, 1, 2, 4, 8, 16, and 32 plants per 6.1 m of row in the center two rows of each plot, which is equivalent to 0, 0.16, 0.33, 0.65, 1.31, 2.62, and 5.25 plants per m of row. The outer two rows of each plot were left as weed-free borders.

Corn and cotton heights were measured at 11, 20, 34, 49, 66, 81, and 104 d after planting (DAP) at Clayton, 20, 24, 31, 37, 46, 58, 72, and 95 DAP at Lewiston-Woodville, and 23, 36, 44, 52, 63, 79, and 101 DAP at Rocky Mount. Up to four randomly selected GR corn plants from each plot were measured from soil surface to top of the plant. Four randomly selected cotton plants from the center two rows of each plot were measured for height from the soil surface to the apical meristem. At the end of the growing season, up to four GR corn plants were randomly selected from each plot to measure above-ground dry biomass and kernel set. The remaining GR corn plants were cut at ground level and removed from plots to facilitate cotton harvest. The center two rows of each plot were harvested once with a spindle picker modified for small-plot research.

Statistical Analyses. Data were tested for homogeneity of variance prior to statistical analysis by plotting residuals. Analysis of variance (ANOVA) was performed on GR corn dry biomass, kernel set, and cotton yield loss as a percentage of weed-free yield. Linear,

quadratic, and higher-order polynomial effects of GR corn density were tested by partitioning sums of squares (Draper and Smith 1981). Weed density main effects were tested by error associated with appropriate location by weed density interactions (McIntosh 1983). If significant GR corn density effects were observed, regression analysis was performed. Nonlinear models were used if ANOVA indicated higher-order polynomial effects of GR corn density were more significant than linear effects. Iterations were performed to determine parameter estimates with least sums of squares for all nonlinear models using the Gauss-Newton method via PROC NLIN in SAS (SAS 1998).

The Gompertz equation was fit to plant heights of each species in each plot (Askew and Wilcut 2001; Draper and Smith 1981; Rawlings et al. 1998). Variables in the Gompertz equation are *H*, *a*, *e*, and *T*, which are based on plant height in cm, the upper asymptote for late-season plant height, the base of natural logarithm, and the time in days after planting, respectively, while *b* and *k* are constants. Multivariate analysis of variance (PROC MANOVA; SAS 1998) was conducted on the three estimated parameters for each fitted curve to test for location, weed density, and location by weed density effects.

The rectangular hyperbola (Askew and Wilcut 2001; Cousens 1988) was used to describe density-dependent effects of GR corn on cotton yield loss. Variables in the rectangular hyperbola are *Y*, *a*, *D*, and *i*, which are based on a percent reduction of weed-free yield, the asymptote for percentage yield loss, the weed density per m crop row, and the yield loss per weed as weed density approaches zero, respectively. Coefficients of determination (R^2) were calculated for nonlinear regressions as in other studies (Askew and Wilcut 2001; Jasieniuk et al. 1999). The approximated R^2 and residual mean squares were used to determine goodness of fit to nonlinear models.

RESULTS AND DISCUSSION

GR Corn and Cotton Height. GR corn and GR cotton heights were significantly different at each location, thus data are presented by location. Heights of GR corn and GR cotton plotted against time fit the Gompertz growth model well (Figure 1). Average late-season height of GR corn was 248, 231, and 234 cm at Clayton, Lewiston-Woodville, and Rocky Mount, respectively. GR corn began to grow taller than GR cotton as early as 25 DAP, depending on location. In addition, cotton height decreased with increasing GR corn density (Figure 2). When grown in competition with 5.25 GR corn plants per m of row, cotton height was reduced by 49, 24, and 28% at Clayton, Lewiston-Woodville, and Rocky Mount, respectively, compared to weed-free cotton (Figure 1). Weeds that grow above crop canopies often intercept light and are more competitive. In addition to direct influences on competition, tall weeds that canopy over cotton may interfere with agrichemical deposition onto cotton foliage. Consequently, yield reduction could be magnified due to indirect influences of weeds that grow taller than cotton like GR corn.

GR Corn Above-Ground Dry Biomass and Seed Production. The effect of GR corn density on GR corn dry biomass was significantly affected by location. Thus data are shown by locations (Figure 3). Glyphosate-resistant corn above-ground dry biomass decreased linearly with increasing weed density at Clayton. At Clayton, GR corn dry biomass decreased from 515 g per plant at 0.16 plants/m of row to 379 g per plant at 5.5 plants/m of row. Buffalobur (*Solanum rostratum* Dunal.) (Rushing et al. 1985a), tropic croton (*Croton glandulosus* var. *septentrionalis* Muell.-Arg.) (Askew and Wilcut 2001),

tumble pigweed (*Amaranthus albus* L.) (Rushing et al. 1985b), and velvetleaf (*Abutilon theophrasti* Medicus) (Bailey et al. 2003) produced 325, 154, 268, and 177 g dry biomass per plant, respectively at 0.2 plants/m density. Density-dependent decline in weed dry biomass per plant is indicative of intraspecific competition (Bridges and Chandler 1987; Rushing et al. 1985a, 1985b; Snipes et al. 1982). However, at Lewiston-Woodville and Rocky Mount, GR corn above-ground biomass was not linearly influenced. The use of hybrid corn may have contributed to the lack of response of corn dry biomass to planting density. Planting densities for corn production generally range from 4.9 to 6.9 plants per m of corn row in North Carolina, depending on row spacing and water holding capacity (Heiniger et al. 2005).

The effect of GR corn density on GR corn kernel production was affected by location, thus data were shown by locations (Figure 4). There was an inverse relationship between GR corn kernel biomass and weed density at Clayton. At Clayton, GR corn kernel biomass decreased from 324 g per plant at 0.16 plants/m of row to 145 g per plant at 5.5 plants/m of row. Similarly to GR corn above-ground dry biomass, the lack of response for seed production at Lewiston-Woodville and Rocky Mount may be explained by the use of hybrid corn and its corresponding recommended planting density.

Cotton Yield Loss. As GR corn dry biomass/m of crop row increased, cotton lint yield decreased (Figure 5). The relationship of dry biomass and lint yield varied between locations. Cotton lint yield decreased 57, 54, and 145 kg/ha at Clayton, Lewiston-Woodville, and Rocky Mount, respectively, with each 500 g increase in weed biomass/m of crop row (Figure 4). At Rocky Mount, GR corn dry biomass remained relatively constant (Figure 3), which may explain the greater reduction in yield with limited increases

in biomass production. Jimsonweed (*Datura stramonium* L.) (Scott et al. 2000), ladysthumb (*Polygonum persicaria* var. *persicaria* L.) (Askew and Wilcut 2002a), Palmer amaranth (*Amaranthus palmeri* L.) (Rowland et al. 1999), Pennsylvania smartweed (*Polygonum pensylvanicum* var. *laevigatum* Fern.) (Askew and Wilcut 2002b), tropic croton (Askew and Wilcut 2001), unicorn-plant [*Proboscidea louisianica* (Mill.) Thellung] (Riffle et al. 1989), and velvetleaf (Smith et al. 1990) also exhibited an inverse relationship of plant biomass to cotton lint yield.

Although *i* values varied from 5 to 9 among locations (Figure 6), concomitant changes in a values were such that predicted lint yield losses at GR corn densities below two plants per m of row were relatively stable. For example, one GR corn plant/m of crop row decreased lint yield 8, 5, and 5% in Clayton, Lewiston-Woodville, and Rocky Mount, respectively. Prediction accuracy at the lower end of weed density ranges is more important than at higher weed densities since economic thresholds often occur at weed densities below one weed/m of crop row (White and Coble 1997). Lee et al. (2005) evaluated GR soybean as a weed in GR cotton with i values ranging from 1.7 to 2.8, depending on location. Byrd and Coble (1991) reported between 1 and 6% lint yield loss, depending upon year, with 0.33 large crabgrass plants per m of crop row. One johnsongrass [*Sorghum halepense* (L.) Pers.] plant per m of cotton row reduced lint yield by 56 and 86% in 1996 and 1997, respectively, when harvested with a spindle picker (Wood et al. 2002). Bridges and Chandler (1987) reported yield losses of 14 and 40% with johnsongrass densities of 0.4 and 0.8 plant per m, respectively. Brown et al. (1985) investigated the influence of bermudagrass [*Cynodon dactylon* (L.) Pers.] interference during an initial establishment season and a subsequent season. Yield losses were only

observed in the initial season with moisture stress during the cotton fruiting period (Brown et al. 1985). However, in subsequent years, yield was negatively affected. For example, one plant per 7.5 m of cotton row reduced yield at least 25% at all locations (Brown et al. 1985). Even though limited data are available for annual grass weed interference, interference of numerous broadleaf weeds in cotton has been investigated. Cotton lint yield losses ranged from 22 to 69% from a weed density of 1 plant/m of row for ivyleaf morningglory, jimsonweed, ladysthumb, pale smartweed, Palmer amaranth, Pennsylvania smartweed, tropic croton, and velvetleaf (Askew and Wilcut 2002a, 2002b, 2002c; Bailey et al. 2003; Morgan et al. 2001; Rogers et al. 1996; Rowland et al. 1999; Scott et al. 2000; Wood et al. 1999). Yield losses associated with GR corn were less than with many grass and broadleaf weeds common in cotton but still significant due to value of the cotton crop. Furthermore, these yield loss estimates may be overestimated due to the use of hybrid corn. Jugenheimer (1976) discussed several characteristics of hybrid vigor. When hybrids are open pollinated, hybrid vigor is reduced (Jugenheimer 1976). In normal field situations with volunteer GR corn, these volunteers would display reduced vigor compared to commercial hybrids.

Numerous graminicides, including clethodim, fluazifop, quizalofop, and sethoxydim, are registered for POST treatment of GR corn control in GR cotton (York and Culpeper 2005). Herbicide costs as listed in HADSS[3] plus a $10/ha application fee are shown (Table 2). Economic threshold was based on a support price of $1.32/kg for cotton lint

[3] HADSS, Herbicide Application Decision Support System-North Carolina version, AgRenaissance Software LLC, PO Box 91235, Raleigh, NC 27675.

(Askew and Wilcut 2001) and weed-free yield potential of 1,704, 1,668, and 1,577 kg/ha at Clayton, Lewiston-Woodville, and Rocky Mount, respectively. The economic threshold for the various graminicides ranged from 2 to 8 GR corn plant per 100 m of crop row (Table 2), depending on herbicide selection and weed-free yield potential. However, these calculations assume that other cotton cultivars will respond similarly to GR corn interference, that graminicides are equally efficacious, that similar weed-free yields are attainable, and a selling price of $1.32/kg for cotton lint.

GR corn is less competitive than many grass and broadleaf weeds of cotton. In addition to direct yield losses, GR corn may limit light interception, interfere with agrichemical applications, and reduce harvest efficiency. Due to known differences in hybrid vigor between commercial hybrids and open-pollinated hybrids (Jugenheimer 1976), these data may overestimate the potential for corn to cause yield losses due to the use of hybrid corn in this study.

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Table 1. Regression parameters ($H = a\ e^{-be-Kt}$) describing the relationship between cotton height and time in days after crop planting at various densities of corn interference where *H*, *a*, e, and *t* are based on the plant height in cm, the upper asymptote for late-season plant height, the base of natural logarithm, and the time in days after planting, respectively, while *b* and *k* are constants. Values in parenthesis are standard errors.

Location	Interference level	*a*	*b*	K	R^2
		Cotton height (cm)			
Lewiston-Woodville	0	93.9 (2.14)	8.55	0.067	0.99
	0.16	95.1 (3.67)	8.75	0.067	0.96
	0.33	94.3 (1.95)	7.25	0.060	0.99
	0.66	88.9 (3.42)	7.67	0.063	0.96
	1.31	83.4 (3.92)	7.68	0.065	0.94
	2.62	72.1 (2.98)	7.33	0.069	0.94
	5.25	71.4 (2.81)	6.43	0.064	0.95
Rocky Mount	0	99.9 (4.02)	9.84	0.064	0.95
	0.16	103.4 (3.45)	9.21	0.060	0.97
	0.33	99.4 (3.39)	9.32	0.062	0.97
	0.66	97.7 (3.19)	8.94	0.062	0.97
	1.31	93.4 (4.06)	9.11	0.063	0.94
	2.62	73.2 (4.86)	8.55	0.068	0.91
	5.25	71.7 (4.86)	8.07	0.066	0.82
Clayton	0	115.4 (5.79)	8.30	0.062	0.95
	0.16	118.7 (5.76)	9.58	0.064	0.95
	0.33	115.7 (7.39)	6.32	0.053	0.93
	0.66	113.1 (5.58)	5.42	0.050	0.96
	1.31	98.0 (4.69)	5.86	0.056	0.95

Table 1 (continued)

	2.62	91.3 (7.13)	4.18	0.048	0.89
	5.25	58.6 (5.12)	4.81	0.073	0.71
		Corn height (cm)			
Lewiston-Woodville		249.2 (4.53)	12.26	0.066	0.96
Rocky Mount		249.5 (5.36)	20.6	0.070	0.93
Clayton		254.9 (3.46)	15.6	0.075	0.97

Table 2. Economic thresholds for glyphosate-resistant corn in glyphosate-resistant cotton.

Herbicide[a]	Cost	Clayton	Lewiston-Woodville	Rocky Mount	Clayton	Lewiston-Woodville	Rocky Mount
	$/ha	– corn plants/100 m of row –			—— plants/ha ——		
Clethodim	35.37	5.05	5.26	2.92	523	546	318
Fluazifop	28.49	4.05	4.22	2.34	419	438	256
Quizalofop	50.04	7.19	7.52	4.15	745	777	453
Sethoxydim	28.15	4.00	4.18	2.31	415	433	253

[a] Herbicide costs included the herbicide (HADSS price) and application costs ($10/ha).

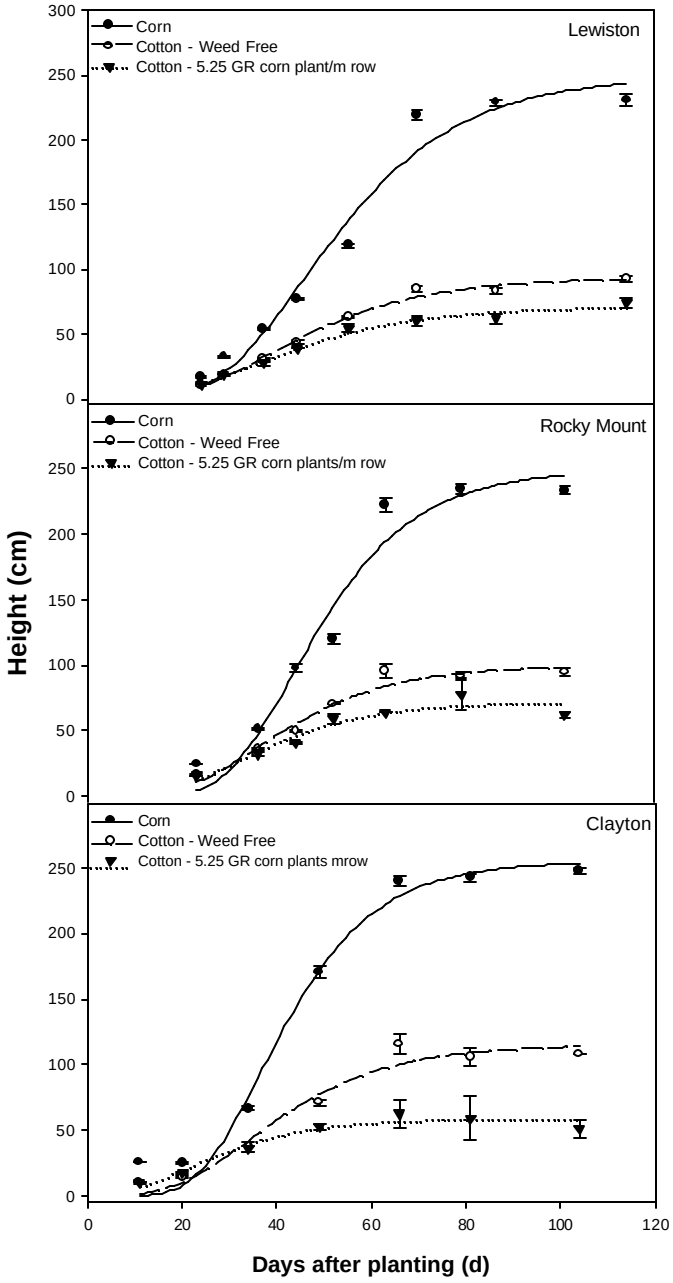

Figure 1. Glyphosate-resistant corn height, glyphosate-resistant cotton height with no weed interference, and glyphosate-resistant cotton height with 5.25 corn plant per m of cotton row are shown. Bars represent standard error of the mean. Regression parameters and corresponding R^2 values are shown in Table 1.

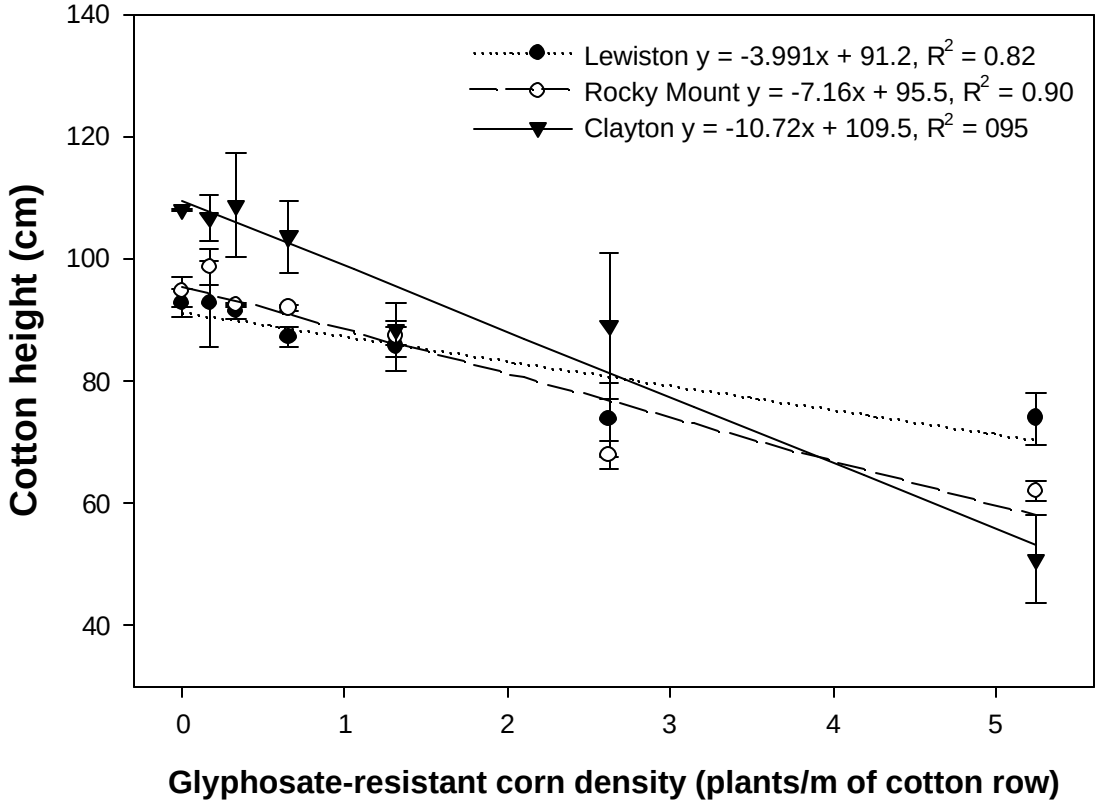

Figure 2. Effect of glyphosate-resistant corn density at the last measuring timing at each location (104, 95, and 101 d after planting at Clayton, Lewiston-Woodville, and Rocky Mount, respectively). Bars represent standard error of the mean.

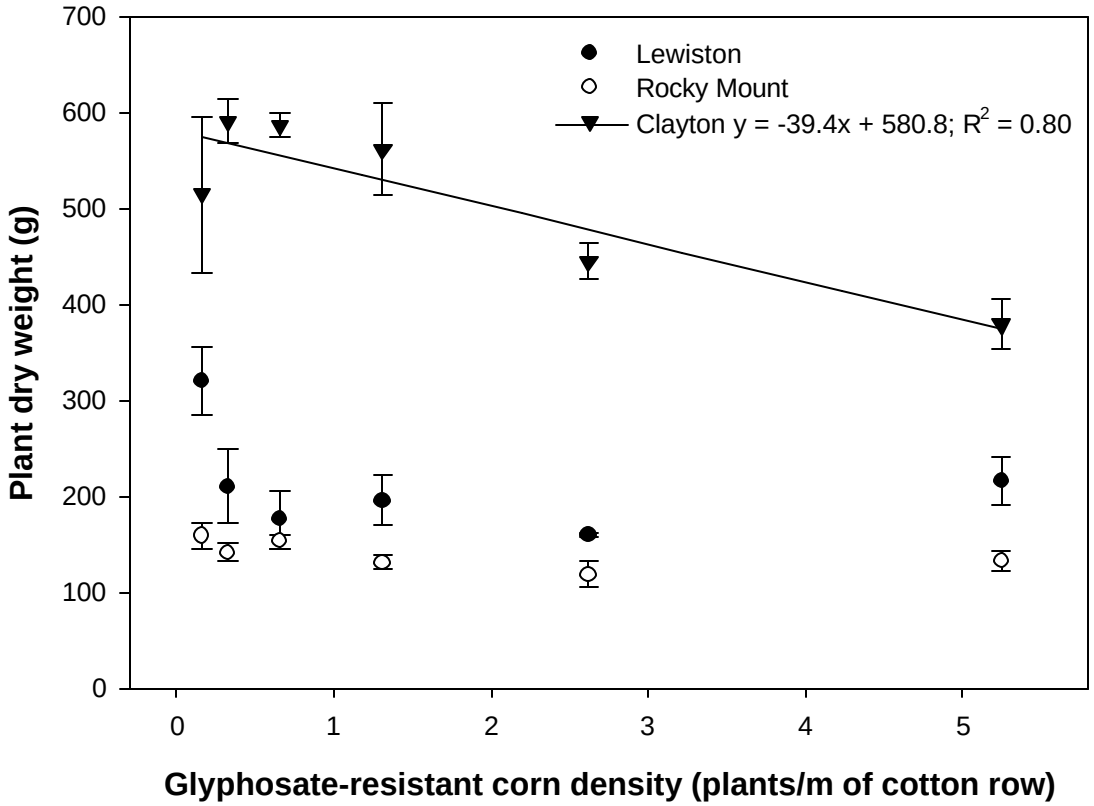

Figure 3. Effect of glyphosate-resistant corn density on late-season glyphosate-resistant corn biomass per plant shown by location. Bars represent standard error of the mean. A linear response was not observed at Lewiston-Woodville and Rocky Mount.

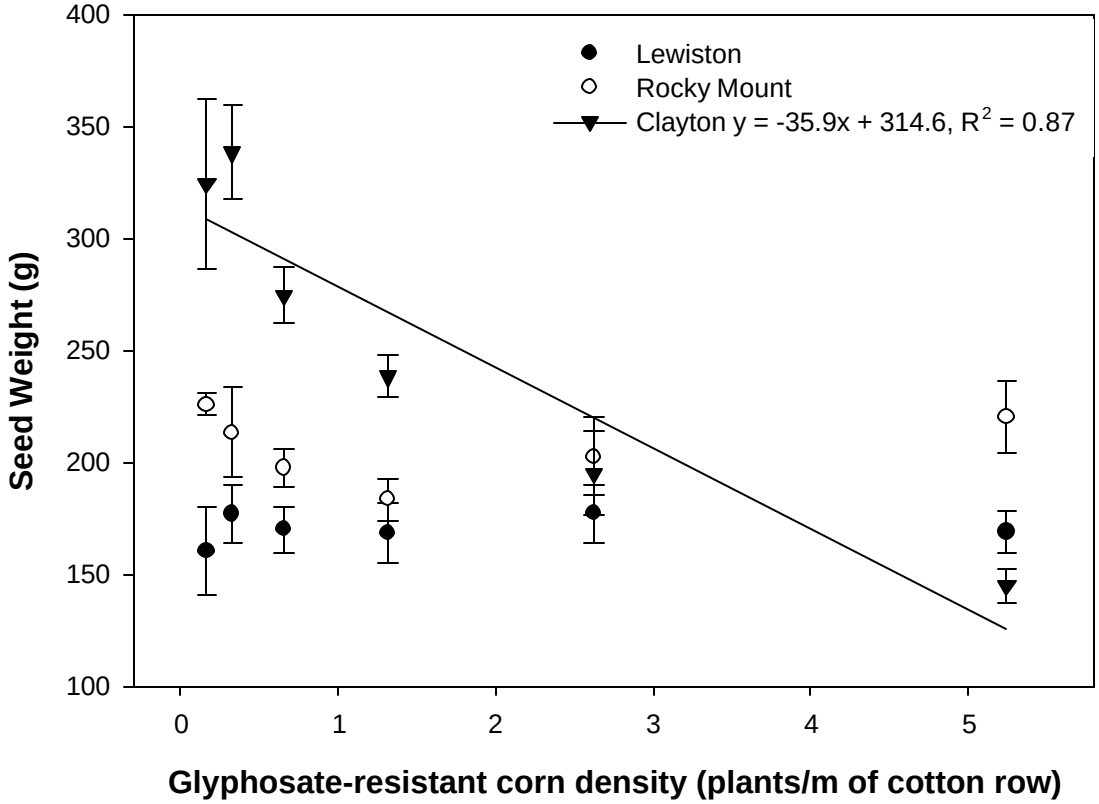

Figure 4. Effect of glyphosate-resistant corn density on late-season glyphosate-resistant corn seed weight per plant shown by location. Bars represent standard error of the mean. A linear response was not observed at Lewiston-Woodville and Rocky Mount.

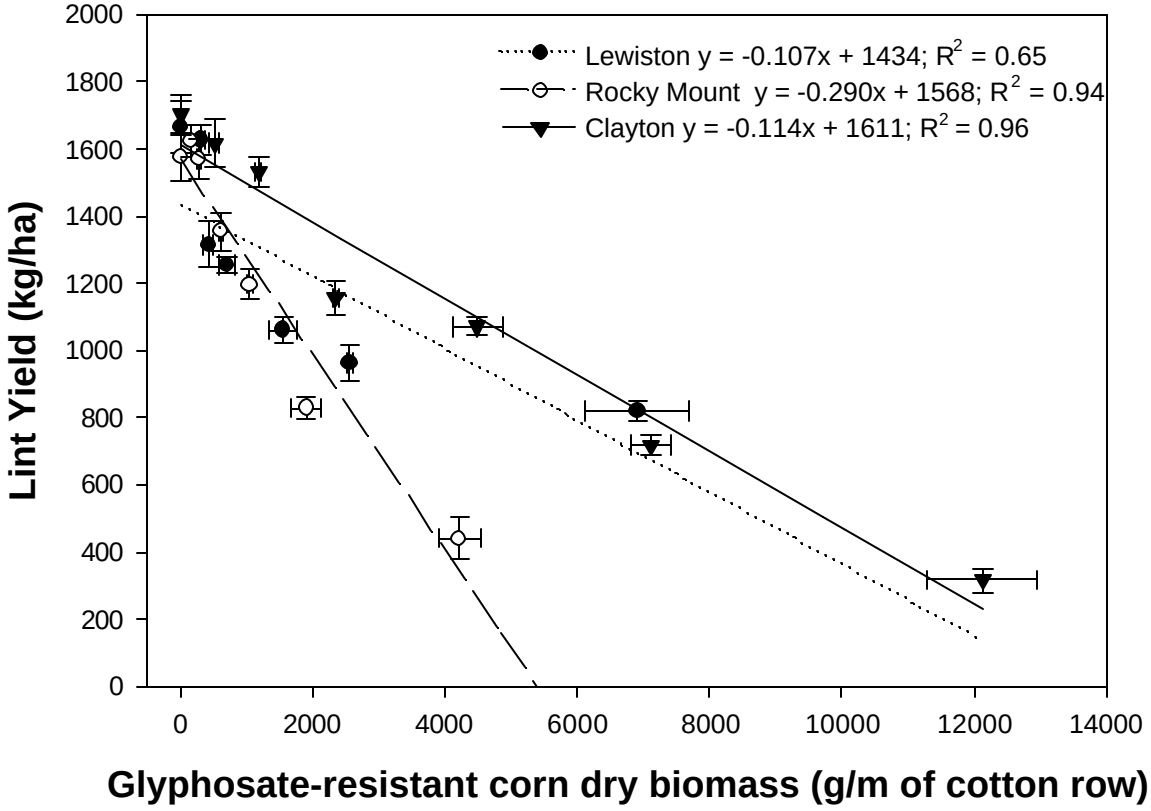

Figure 5. Effect of glyphosate-resistant corn biomass per m crop row on cotton lint yield shown by location. Bars represent standard error of the mean.

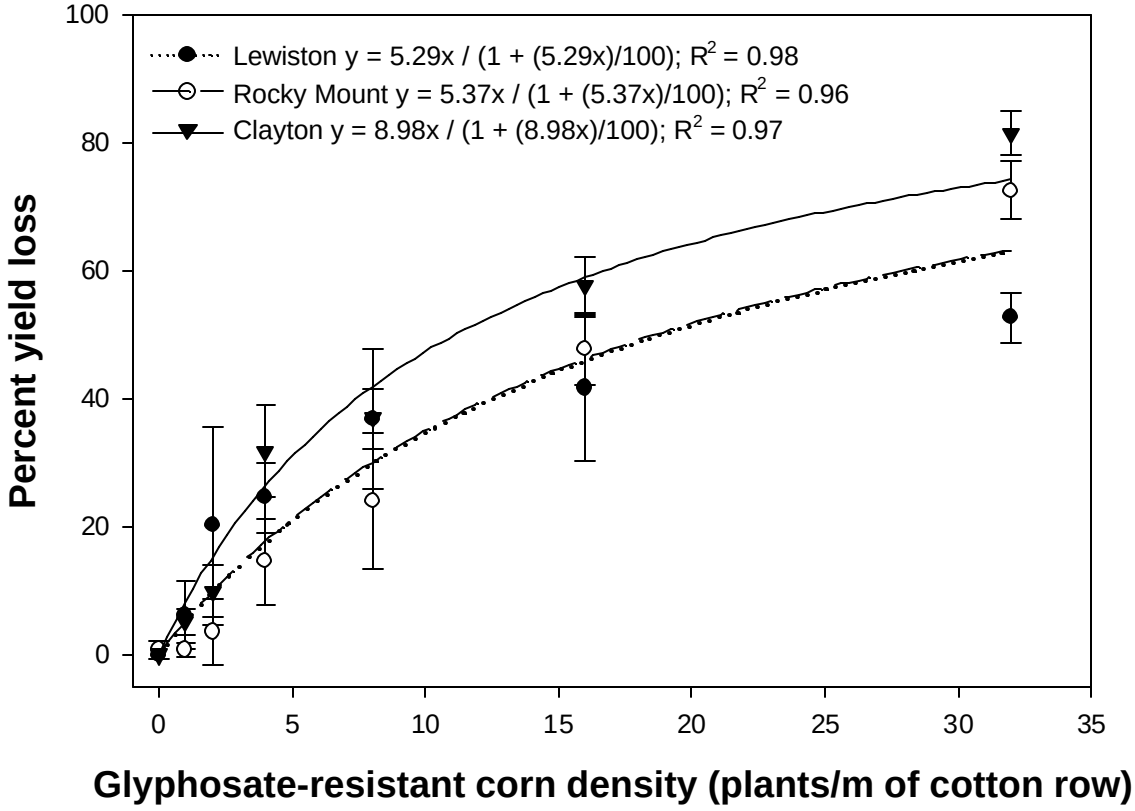

Figure 6. Cotton lint yield loss associated with season-long glyphosate-resistant corn interference. Bars represent standard error of the mean.

Influence of corn growth stage on absorption and translocation of glyphosate in glyphosate-resistant corn

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Abstract: Two studies were conducted to investigate the influence of corn growth stage on absorption and translocation of glyphosate in glyphosate-resistant (GR) corn. In the first study, GR corn was treated with ^{14}C-glyphosate at three growth stages and harvested at 3 and 7 d after treatment (DAT). Averaged over trials, growth stages, and harvest timings, 42% of the applied ^{14}C-glyphosate was absorbed. Regardless of corn growth stage, the leaves above the treated leaf were the greatest sinks for ^{14}C-glyphosate, accumulating 8% of the applied ^{14}C-glyphosate. Root tissue also accumulated significant levels of glyphosate (5%) compared to other potential sites of glyphosate translocation. A second study was conducted to determine the distribution of ^{14}C-glyphosate at V4, V6, and V8 growth stages when harvested at V8, V12, V16, and R1 stages. Forty-four to 60% of the applied ^{14}C-glyphosate remained in the corn tissues at anthesis. The leaves above the treated leaf and roots accumulated the greatest amounts of ^{14}C-glyphosate, regardless of corn growth stages. When plants were treated at V4, V6, and V8 stages, the concentration of ^{14}C-glyphosate in the tassel at the V12 harvest timing was 184, 431, and 921 Bq g^{-1} dry tissue, respectively. Likewise, increasing levels of ^{14}C-glyphosate concentrations between corn growth stages were also observed in ear shoots. These data suggest that reproductive tissues such as the tassel and ear shoots can accumulate ^{14}C-glyphosate at higher concentrations than other tissues, especially when the herbicide is applied POST after the V6 stage.

Nomenclature: Glyphosate; corn 'DK 687 RR'.

Keywords: Glyphosate distribution, herbicide-resistant crops, transgenic crops.

Introduction

Since their commercial release in the mid 1990s, GR crops have become extremely popular among growers due to reduced potential for environmental impact (Sprankle et al. 1975; Wauchope et al. 2002), broad-spectrum weed control (Corbett et al. 2004; Culpepper and York 1998; Culpepper et al. 2000; Johnson et al. 2000; Payne and Oliver 2000; Scott et al. 2002), and postemergence (POST) application flexibility (Hart and Wax 1999; Johnson 2000). These benefits are also apparent in GR corn, where glyphosate can be applied POST up to the V8 stage or 76 cm in height (Anonymous 2003), but adoption of GR corn has been slower when compared to GR cotton (*Gossypium hirsutum* L.) and soybean [*Glycine max* (L.) Merr.] (Anonymous 2005).

Glyphosate has reportedly caused yield reduction in GR cotton (Jones and Snipes 1999; Viator et al. 2004). Further investigations concluded that yield reductions were due to lower CP4-5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) expression in male reproductive portions of cotton flowers compared to vegetative tissues (Pline et al. 2002a). In addition to lower EPSPS levels, pollen viability was reduced and stigma length was increased following glyphosate treatments (Pline et al. 2002a, 2002b, 2002c; Yasour et al. 2000). Even though no yield reductions have been reported in GR corn (Ferrell and Witt 2002; Johnson et al. 2000; Nolte and Young 2002) and soybean (Elmore et al. 2001a, 2001b) in response to glyphosate treatments, research was conducted to evaluate reproductive responses in GR corn, including pollen viability and yield potential (Thomas et al. 2004). Pollen viability reductions were evident with glyphosate treatments after the V6 stage, however, no yield reductions were observed with any glyphosate treatment combination (Thomas et al. 2004). Since tassel initiation can begin as early as 2 wks after

emergence (Kiesselbach 1992), it is possible that any glyphosate treatment to GR corn after tassel initiation may negatively affect pollen viability, and anther and pollen production. Studies have shown that glyphosate translocation is similar to photoassimilate translocation (Gougler and Geiger 1981; McAllister and Haderlie 1985), which follows a source to sink relationship (Sandberg et al. 1980; Wyrill and Burnside 1976). More recent research demonstrated glyphosate accumulation in sinks, including meristematic and reproductive regions (Feng et al. 2003; Hetherington et al. 1999; Pline et al. 2001; Viator et al. 2003). Therefore, it is probable that glyphosate may accumulate in reproductive sinks like the ear and tassel. If accumulation does occur, then this accumulation may explain the resulting pollen damage and reduction in pollen and anther production (Thomas et al. 2004).

Our objectives were to evaluate absorption and translocation of glyphosate applied at the V4, V6, or V8 stage using various harvest timings. The first study evaluated the immediate effects (3 and 7 DAT) on absorption and translocation of glyphosate. A second study evaluated the distribution of foliar-applied glyphosate by harvesting at four vegetative and reproductive stages.

Materials and Methods

Plant material. For each study, Dekalb '687 RR' GR corn was planted in 30-cm diameter pots containing a commercial potting mixture[1] and grown in a polyethylene covered greenhouse maintained at 25 ± 2 C constant temperature where natural sunlight was supplemented 4 h daily with mercury halide lights, providing a 16-h day length. Studies were conducted from August 2002 to April 2003.

^{14}C-Glyphosate Treatments and Sampling. Glyphosate at 0.84 kg ae ha^{-1} was applied POST at V4, V6, and V8 corn growth stages. Before each glyphosate POST application, a 20-cm^2 area of the newest completely expanded leaf was covered with aluminum foil to prevent spray contact with the leaf surface. Ten 1-? l droplets of ^{14}C-glyphosate[2] containing 5 kBq plus 0.25% (v/v) non-ionic surfactant[3] were manually applied with a microsyringe to the area of each covered leaf portion in the ^{14}C-glyphosate timing study (Pline et al. 2001). In the ^{14}C-glyphosate-corn growth stage study, 16.7 kBq of ^{14}C-glyphosate plus 0.25% (v/v) non-ionic surfactant were applied as previously described.

For the timing study, plants were harvested at 3 and 7 d after treatment (DAT). Plants in the glyphosate-corn growth stage study were harvested at V8, V12, V16, and R1. Absorption was determined by rinsing the treated leaf portion with 10 ml of a methanol:water (1:1, v/v) plus 0.25% (v/v) non-ionic surfactant to remove non-absorbed glyphosate (Askew and Wilcut 2002; Pline et al. 2001). A 1.0-ml aliquot was taken from the leaf rinsate, diluted in 15 ml scintillation fluid[4] and radioactivity was quantified with liquid scintillation spectrometry (LSS)[5]. All plants were then divided into four regions: 1) treated leaf, 2) above treated leaf, 3) below treated leaf, and 4) roots. The treated leaf was removed at the point of attachment to the stem, which determined the division for above and below the treated leaf sections. The treated leaf was divided into treated portion, tip, base, and sheath. The above-treated and below-treated leaf tissues were each divided into leaves and stem. If present, the developing brace roots, tassel, and ear shoots were removed and placed in separate bags. Plant parts were dried for 48 h at 70 C, weighed, homogenized, and combusted with a biological sample oxidizer[6]. Radioactivity in the oxidized samples was quantified by LSS.

Experimental Design and Data Analysis. The glyphosate timing study was arranged as factorial with glyphosate application timing and harvest interval factors in a completely randomized design with four replications. The glyphosate-corn growth stage study was also arranged as a factorial with glyphosate application timing and harvest interval factors in a completely randomized design with four replications. Both studies were repeated once in time. Prior to analysis, data were tested for homogeneity of variance by plotting residuals. Log transformation of 14C-glyphosate concentration (Bq g^{-1} dry tissue) improved homogeneity of variance based on visual inspection of plotted residuals; therefore, data were transformed. Analysis of variance conducted using a mixed model in SAS[8] revealed no run by treatment interaction, thus data were averaged over runs. Data were analyzed for main effects and interactions. Mean separations were performed on data using Fisher's Protected LSD at P=0.05. When interactions were significant, LSD tests were performed separately across the levels of a given factor within the levels of the other factor.

Results and Discussion

^{14}C-Glyphosate Timing Study. The objective of this study was to measure the amount of ^{14}C-glyphosate, applied POST at V4, V6, and V8 corn growth stages, remaining in the plant tissues at 3 and 7 DAT. Absorption of glyphosate was not influenced by trial, corn growth stage, or harvest timing, thus data were averaged over trial, growth stage, and harvest timing. Forty-two percent of the applied ^{14}C-glyphosate was collected in the leaf wash (Table 1). Therefore, these data suggest that absorption of ^{14}C-glyphosate had reached a plateau at 3 DAT. Previous research has indicated that glyphosate absorption is

biphasic, with an initial rapid rate of glyphosate absorption in the first 24 h followed by a longer phase of slow uptake (Burton and Balke 1988; Feng et al. 2003; Gaskin and Holloway 1992; Hetherington et al. 1999; Masiunas and Weller 1988). When glyphosate was applied in fine, medium, and coarse droplet sizes, Feng et al. (2003) reported that 30, 35, and 49% of the applied ^{14}C-glyphosate was absorbed at 3 DAT, respectively. Similarly, Hetherington et al. (1999) reported between 45 to 65% absorption of the applied ^{14}C-glyphosate in corn, depending on glyphosate concentration.

Translocation of ^{14}C-glyphosate as a percentage of applied was not influenced by trial, treatment timing, or harvest timing, thus data were averaged over trial, treatment timing, and harvest timing (Table 1). Regardless of corn growth stage, the leaves above the treated leaf were the greatest sinks for ^{14}C-glyphosate, accumulating 8% of the applied ^{14}C-glyphosate. Root tissue also accumulated significant levels of glyphosate (5%) compared to other potential sites of glyphosate translocation. All other plant parts accumulated 1.4% or less of applied ^{14}C-glyphosate. Feng et al. (2003) observed 9 to 16% and 12 to 23% of the recovered ^{14}C-glyphosate in roots and leaves 6 to 8 of GR corn, respectively, depending on the droplet size. Hetherington et al. (1999) reported that the shoot and root fractions accumulated 25 to 40% of the applied ^{14}C-glyphosate. Previous research in GR cotton (Pline et al. 2001; Viator et al. 2003) and weed species (Kirkwood et al. 2000; Sandberg et al. 1980; Satchivi et al. 2000) have shown similar source to sink translocation patterns with glyphosate.

The concentration of ^{14}C-glyphosate as activity per unit of dry matter (Bq g^{-1} dry tissue) was not influenced by trial or harvest timing, thus data were averaged over trial and harvest timing (Table 2). The concentration of translocated ^{14}C-glyphosate was not

significantly different at either harvest timing. However, significant levels of accumulation were observed for several plant parts compared across corn growth stages. Concentration of ^{14}C-glyphosate in the leaves above and below the treated leaf and the sheath and tip of the treated leaf declined with increasing growth stage (Table 2). The decline in concentration in the leaves may be an indication of a shift in sink strength. Kiesselbach (1992) has indicated that tassel and ear formation can begin as early as the V6 stage. Even though tassels were not found at the V6 stage, tassels at the V8 stage contained numerically 3 and 6 times greater concentration of ^{14}C-glyphosate than leaves above the treated leaf and roots, respectively. These levels of glyphosate accumulation in the tassel may explain reduced pollen viability due to glyphosate application after the V6 stage (Thomas et al. 2004).

^{14}C-Glyphosate-Corn Growth Stage Study. The objective of this study was to measure the amount of ^{14}C-glyphosate, applied POST at V4, V6, and V8 growth stages, remaining in the plant tissues at various growth stages up to anthesis. The level of ^{14}C-glyphosate recovery was variable and never exceeded 74% of the applied (Table 3). In a similar evaluation of ^{14}C-glyphosate fate in GR cotton, Pline et al. (2001) reported recovery levels ranging from 36 to 99% of the applied, depending on the application and harvest timings. McAllister and Haderlie (1985) reported recovery averaging 42% of the applied ^{14}C-glyphosate in an outdoor study in which the ^{14}C-glyphosate label was left on treated Canada thistle [*Cirsium arvense* (L.) Scop.] plants for 8 d. ^{14}C-glyphosate degradation either by the plant or by leaf surface microbes with a subsequent loss of $^{14}CO_2$ may explain low ^{14}C recovery (McAllister and Haderlie 1985; Pline et al. 2001). Additionally, translocation of ^{14}C-glyphosate beyond the region in which roots were collected due to

harvest inefficiency or exudation may also be potential sources of losses (McAllister and Haderlie 1985; Pline et al. 2001; Rodrigues et al. 1982).

At all corn growth stages, accumulation based on the percentage of applied ^{14}C-glyphosate was greatest in the leaves above the treated leaf and roots (Table 3), regardless of growth stage or harvest timing. After accounting for significant differences in biomass of the various plant parts (Table 4), the concentration of ^{14}C-glyphosate accumulated (Bq g^{-1} dry tissue) in these leaves is similar to other vegetative tissues. However, roots and reproductive tissue show numerically higher concentrations of ^{14}C-glyphosate compared to the leaves above the treated leaf at V8, V12, and V16 harvest timings, regardless of growth stage (Table 4). Furthermore, the concentration of ^{14}C-glyphosate in the roots of plants treated at V4 declined from 444 to 168 Bq g^{-1} with harvests at V8 and R1, respectively. When ^{14}C-glyphosate was applied at V6 and V8 stages, similar numerical reductions of ^{14}C-glyphosate concentrations were observed in roots. The decline in translocation to vegetative sinks may indicate a shift toward reproductive development. Pline et al. (2001) observed a similar alteration in translocation pattern comparing vegetative and reproductive stages of cotton. Following cessation of fruiting in cotton, resources (^{14}C) were reallocated to roots, which may be influenced by the perennial habitat of cotton (Pline et al. 2001). Since corn is annual, a similar reallocation of ^{14}C to the roots was not observed.

As accumulation in vegetative tissues began to decline through successive harvests, a corresponding increase in accumulation of ^{14}C-glyphosate was observed in tassels and ear shoots (Table 3 and 4). The observed glyphosate translocation patterns resemble normal translocation patterns of plants shifting from vegetative to reproductive stages of growth.

In plants treated at the V4 stage, the percentage of applied ^{14}C-glyphosate in reproductive tissues (sum of percentages for tassel and ear shoots) was 0.01, 0.37, 1.06, and 0.87% at V8, V12, V16, and R1 harvest timings, respectively. When glyphosate was applied POST to corn at the V8 stage, accumulation in reproductive tissues was 0.03, 1.59, 4.88, and 2.06% at V8, V12, V16, and R1 harvest timings, respectively. At the time of the R1 harvest, significant amounts of pollen had been shed, which may be responsible for the observed decline in ^{14}C-glyphosate accumulation.

The concentration of ^{14}C-glyphosate (Bq g^{-1} dry tissue) also increased in reproductive tissues. Numerical increases of ^{14}C-glyphosate concentrations in the tassel and ear shoots compared to above ground vegetative tissues were apparent, regardless of growth stage. When plants were treated at V4, V6, and V8 stages, the concentration of ^{14}C-glyphosate in the tassel at the V12 harvest timing was 184, 431, and 921 Bq g^{-1} dry tissue, respectively. Similarly, the concentration of ^{14}C-glyphosate in ear shoots was the greatest at the V12 harvest timing with 253, 464, and 1,166 Bq g^{-1} dry tissue in plants treated at the V4, V6, and V8 stages, respectively. Even though these are numerical differences, the stage-dependent differences in ^{14}C-glyphosate concentration in the tassel may explain the observed reductions in corn pollen viability in response to glyphosate POST treatments after the V6 stage (Thomas et al. 2004). Based on these data, glyphosate POST treatments made later in the corn growing season result in greater glyphosate accumulation in reproductive tissues.

Forty-four to 60% of the applied ^{14}C-glyphosate remained in the corn tissues at anthesis. In addition to glyphosate remaining in the plant tissue through anthesis, glyphosate was transported to meristematic regions including apical leaves and stems,

roots, and reproductive tissues. However, the level of glyphosate translocation to reproductive tissues was dependent on the corn growth stage at the time of glyphosate treatment with the greatest accumulation in plants treated at the V8 stage. In general, these data offer some explanation into the observed growth stage dependent reproductive response - specifically pollen viability to glyphosate POST treatments in GR corn (Thomas et al. 2004).

Sources of Materials

[1] MetroMix 200, Sun Gro Horticulture, 15831 N.E. 8th Street, Suite 100, Bellevue, WA 98008.

[2] Roundup UltraMax, Monsanto Co., 800 N. Lindbergh Blvd., St. Louis, MO 63167.

[3] ^{14}C-glyphosate G-8392, Sigma Chemical Co., P. O. Box 14508, St. Louis, MO 63178.

[4] Induce (mixture of alkyl polyoxylkane ether, free fatty acids, and isopropanol), Helena Chemical Co., 5100 Popular Avenue, Memphis, TN 38137.

[5] Scintiverse® SX18-4 Universal Liquid Scintillation Cocktail, Fisher Scientific, 1 Regeant Road, Fair Lawn, NJ 07410.

[6] Packard Tri-Carb 2100TR Liquid Scintillation Spectrometer, Packard Instrument Co., 220 Warrenville Rd., Downers Grove, IL 60515.

[7] Model OX500 Biological Material Oxidizer, R. J. Harvey Instrument Corp., 123 Patterson St., Hillsdale, NJ 07642.

[8] [SAS] Statistical Analysis Systems software, Ver. 8., SAS Institute, Inc., Box 8000, SAS Circle, Cary, NC 27513.

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Table 1. Distribution of ^{14}C-glyphosate in glyphosate-resistant corn, averaged over trials, treatment timings (V4, V6, and V8), and harvests timings (3 and 7 d after treatment).[a]

Plant Part	Mean[bc]	SEM
	— % of ^{14}C glyphosate applied —	
ATL-leaves	7.8	0.4
BTL-leaves	1.4	0.1
BTL-stems	1.3	0.4
Roots	4.8	0.3
Tassel[d]	0.1	0.0
TL	5.3	0.5
TL-base	1.2	0.2
TL-sheath	0.9	0.1
TL-tip	0.9	0.2
Leaf wash	41.5	1.5
LSD[b]	1.72	

[a] Abbreviations: ATL, above treated leaf; BTL, below treated leaf; SEM, standard error of the mean; TL, treated leaf.

[b] Least significant difference at P=0.05 of translocation data.

[c] Total recovery of ^{14}C-glyphosate was 65.2%, averaged over trials, treatment timings, and harvest timings.

[d] Tassels were only harvested with V8 treatments and harvest timings.

Table 2. Concentration of ^{14}C-glyphosate in glyphosate-resistant corn treated at V4, V6, or V8, averaged over trials and harvests (3 and 7 d after treatment).[a]

Plant Part	Corn Growth Stage						
	V4		V6		V8		LSD[c]
	Mean	SEM	Mean	SEM	Mean	SEM	
	Bq g^{-1} dry tissue						
ATL-leaves	347.04	33.40	108.10	8.58	83.62	4.96	49
BTL-leaves	100.42	10.81	68.03	7.76	18.09	0.62	28
BTL-stems	234.35	36.99	251.59	143.80	31.47	2.19	NS
Roots	253.24	26.69	64.80	4.83	42.86	3.00	NS
Tassel	NA		NA		242.07	32.18	NA
TL	8879.01	937.30	5221.55	1025.20	4448.69	566.18	1958
TL-base	316.28	44.86	231.69	83.35	115.68	18.82	NS
TL-sheath	244.80	29.41	112.88	10.72	88.80	11.08	60
TL-tip	334.30	34.14	181.43	33.48	103.37	7.65	122
LSD[b]	620.7		620.3		289.1		

[a] Abbreviations: ATL, above treated leaf; BTL, below treated leaf; SEM, standard error of the mean; TL, treated leaf.

[b] Least significant difference at P=0.05 of data within columns.

[c] Least significant difference at P=0.05 of data within rows.

Table 3. Distribution of foliar-applied ^{14}C-glyphosate in glyphosate-resistant corn treated at V4, V6, or V8 and harvested at various vegetative and reproductive growth stages, averaged over trials.

Stag	Plant part	Harvest timing								
		V8		V12		V16		R1		
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	LSD[a]
		% of ^{14}C glyphosate applied								
4	ATL-leaves	4.67	1.88	9.22	2.59	8.84	2.46	6.90	1.58	NS
4	ATL - stems	0.46	0.23	1.86	0.46	3.65	1.16	2.67	0.57	NS
4	Brace roots	0.14	0.06	2.89	1.39	2.54	0.78	1.77	0.30	NS
4	BTL - leaves	0.06	0.02	0.12	0.05	0.16	0.06	0.04	0.01	NS
4	BTL - stems	0.07	0.04	.	.	0.03	0.00	0.11	0.11	NS
4	Roots	8.49	1.71	11.88	1.32	10.71	1.32	9.07	2.58	NS
4	Ear shoots	.	.	0.05	0.01	0.27	0.03	0.52	0.12	0.16
4	Tassel	0.01	0.00	0.32	0.12	0.79	0.13	0.35	0.07	0.45
4	TL	3.40	1.56	9.98	1.72	12.60	1.11	11.74	3.41	NS
4	TL - base	0.12	0.06	0.13	0.02	0.21	0.04	0.65	0.23	0.20
4	TL - sheath	0.08	0.03	0.37	0.05	0.37	0.06	0.86	0.42	NS
4	TL - tip	0.49	0.26	0.78	0.19	0.58	0.07	0.54	0.13	NS
4	Leaf wash	23.81	5.45	22.09	5.42	26.60	1.81	8.12	2.36	NS
Recovery		41.80		59.70		67.33		43.67		
6	ATL-leaves	2.61	1.09	16.18	4.44	11.11	4.45	12.63	3.42	NS
6	ATL - stems	0.59	0.22	9.68	6.93	3.76	0.88	7.30	3.96	NS
6	Brace roots	1.12	0.62	3.24	0.19	4.82	1.23	2.42	0.85	2.33
6	BTL - leaves	0.10	0.04	0.21	0.04	0.39	0.09	0.43	0.20	NS
6	BTL - stems	0.39	0.30	1.65	0.23	1.63	0.42	1.30	0.30	NS
6	Roots	8.61	1.84	11.52	1.99	13.09	1.04	10.23	2.39	NS
6	Ear shoots	.	.	0.14	0.04	0.71	0.13	1.52	0.45	NS
6	Tassel	0.01	0.01	0.81	0.13	1.79	0.25	0.33	0.13	0.44

Table 3 (continued)

6	TL	3.81	2.30	15.90	1.01	13.80	1.15	14.77	2.88	5.7
6	TL - base	0.57	0.36	0.77	0.15	1.32	0.49	1.41	0.38	NS
6	TL - sheath	1.89	1.69	1.39	0.34	0.88	0.15	1.95	0.74	NS
6	TL - tip	0.34	0.13	2.19	0.35	2.14	0.61	2.53	0.48	NS
6	Leaf wash	11.92	4.58	3.40	0.75	1.98	0.50	1.67	0.38	NS
Recovery		31.94		67.08		57.42		58.50		
8	ATL-leaves	17.42	6.17	22.49	6.46	14.51	5.00	11.58	3.04	NS
8	ATL - stems	0.57	0.37	3.81	1.41	5.53	1.41	3.72	0.98	NS
8	Brace roots	2.09	1.18	5.17	1.38	7.71	1.61	6.05	2.03	8.23
8	BTL - leaves	0.37	0.02	1.01	0.26	1.46	0.28	0.92	0.24	NS
8	BTL - stems	0.87	0.54	6.83	0.90	6.56	0.54	3.75	0.87	3.54
8	Roots	7.78	1.57	11.61	2.24	11.72	1.59	10.00	2.96	NS
8	Ear shoots	.	.	0.18	0.03	1.07	0.14	1.67	0.32	0.63
8	Tassel	0.03	0.02	1.41	0.32	3.81	0.47	0.39	0.16	1.76
8	TL	8.46	2.47	9.38	1.39	11.49	0.86	9.21	1.95	NS
8	TL - base	0.90	0.25	1.07	0.12	1.41	0.22	3.64	1.12	NS
8	TL - sheath	1.21	0.46	3.39	2.02	3.08	1.84	1.69	0.42	NS
8	TL - tip	1.64	0.58	2.98	0.49	2.77	0.28	6.06	1.77	NS
8	Leaf wash	2.97	0.76	2.01	0.42	2.24	0.27	1.04	0.40	NS
Recovery		44.32		71.35		73.37		59.72		
		5.15		8.06		3.72		4.67		

[a] Least significant difference at P=0.05 of translocation data within columns.

[b] Least significant difference at P=0.05 of translocation data within rows.

Table 4. Distribution of foliar-applied ^{14}C-glyphosate in glyphosate-resistant corn treated at V4, V6, or V8 and harvested at various vegetative and reproductive growth stages, averaged over trials.

Stage	Plant part	Harvest timing								
		V8		V12		V16		R1		
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	LSD[a]
		Bq g^{-1} dry tissue								
4	ATL-leaves	61.36	24.89	66.25	17.82	55.07	15.68	41.01	11.83	NS
4	ATL - stems	21.82	8.75	30.70	8.70	28.79	8.27	14.64	2.41	NS
4	Brace roots	136.07	31.69	169.40	74.88	95.69	21.14	51.39	8.26	NS
4	BTL - leaves	18.90	8.68	71.18	28.69	121.28	57.56	29.12	3.46	NS
4	BTL - stems	25.50	6.10	.	.	.	.	45.05	.	.
4	Roots	444.34	84.52	443.88	54.12	249.04	29.67	167.85	40.07	187
4	Ear shoots	.	.	253.40	52.00	79.39	13.17	20.38	4.83	NS
4	Tassel	371.88	140.49	183.52	38.06	117.62	20.69	42.31	8.45	NS
4	TL	11332.65	4846.67	29233.99	5152.85	39951.42	4328.45	37265.64	13462.13	NS
4	TL - base	158.05	81.59	198.21	23.95	283.06	44.33	534.29	164.51	NS
4	TL - sheath	75.13	34.61	321.09	29.37	317.44	41.69	821.47	473.40	NS
4	TL - tip	537.14	160.28	1239.19	148.05	1240.76	140.27	988.46	217.49	NS
6	ATL-leaves	25.95	9.69	115.80	33.02	65.56	26.40	78.33	23.49	NS
6	ATL - stems	27.68	12.06	157.63	111.29	38.78	8.97	36.66	18.75	NS
6	Brace roots	160.81	42.39	196.53	22.45	172.81	21.66	73.42	26.02	NS
6	BTL - leaves	20.90	4.28	27.60	6.18	34.23	4.66	45.83	21.76	NS
6	BTL - stems	36.99	25.42	164.95	20.95	119.63	15.65	53.64	9.98	NS
6	Roots	417.89	89.10	405.56	62.06	274.48	28.60	188.27	35.34	NS
6	Ear shoots	.	.	464.11	47.46	243.19	37.31	30.07	5.83	92.1
6	Tassel	160.33	36.03	430.76	68.42	240.24	19.48	44.80	18.43	137
6	TL	6881.78	3862.99	24362.96	1674.84	29586.34	2260.07	22986.46	5082.29	7245
6	TL - base	276.00	141.03	285.27	60.60	491.56	169.80	504.71	143.45	NS
6	TL - sheath	1733.03	1626.23	416.04	96.41	292.06	48.48	595.73	248.83	NS

Table 4 (continued)

6	TL - tip	778.19	485.23	1253.20	134.93	1375.61	329.74	1775.92	399.00	NS
8	ATL-leaves	318.01	127.56	202.14	57.09	88.70	30.86	78.41	27.23	NS
8	ATL - stems	178.99	112.21	124.79	46.68	73.64	19.65	28.87	6.43	NS
8	Brace roots	360.75	157.04	272.83	65.58	373.49	54.71	152.07	46.30	NS
8	BTL - leaves	16.93	3.06	37.18	9.61	47.43	9.46	31.67	9.19	NS
8	BTL - stems	43.77	26.29	222.27	19.74	151.57	10.40	53.29	11.93	85.9
8	Roots	344.66	53.61	436.67	66.23	291.08	44.14	152.00	33.95	NS
8	Ear shoots	.	.	1166.40	221.97	301.72	39.48	45.98	6.72	212.3
8	Tassel	1047.09	625.06	921.09	131.67	447.00	54.71	45.74	17.94	NS
8	TL	9358.86	2770.10	11930.08	1780.90	20438.68	2953.29	10402.89	2438.42	NS
8	TL - base	174.79	53.58	186.79	14.93	246.05	41.68	585.81	138.09	NS
8	TL - sheath	333.41	140.09	300.80	38.01	714.01	458.72	435.61	103.90	NS
8	TL - tip	632.23	267.60	1065.47	179.23	859.43	155.12	3689.21	1723.66	1486
LSD[b]		2580		2345		5583		8428		

[a] Least significant difference at P=0.05 of data within columns.

[b] Least significant difference at P=0.05 of data within rows.

Absorption and Translocation of Glyphosate and Sucrose in Glyphosate-Resistant Cotton[4]

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Abstract: Studies were conducted to evaluate absorption and translocation of ^{14}C-glyphosate in glyphosate-resistant (GR) cotton. Both commercial GR cotton events [glyphosate-resistant event 1, marketed as Roundup Ready®– released 1997 (GRE1) and glyphosate-resistant event 2, marketed as Roundup Ready Flex® – released 2006 (GRE2)] were evaluated at the 4-leaf (lf) and 8-lf growth stages. Plants were harvested at 1, 3, 5, and 7 d after treatment (DAT). Glyphosate absorption, as a percentage of applied, increased over time with 29 and 36% absorption at 7 DAT in 4-lf GRE1 and GRE2 cotton, respectively. In 8-lf cotton, glyphosate absorption (33% at 7 DAT) was not different between events. Glyphosate translocation patterns were not different between events or harvest timings and exhibited a source-sink relationship. Observed translocation differences between cotton growth stages were probably due to reduced glyphosate export

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from the treated leaf of 8-lf cotton. An additional study compared absorption and translocation of ^{14}C-glyphosate and ^{14}C-sucrose in 5- and 10-lf GRE2 cotton. Averaged over trials, ^{14}C compounds, and growth stages, cotton absorbed 28% of the applied dose at 14 d after treatment. Based on the percentage of ^{14}C exported out of the treated leaf, glyphosate and sucrose translocation patterns were similar, indicating that glyphosate may be used as a photoassimilate model in GRE2 cotton.

Nomenclature: Glyphosate; cotton, *Gossypium hirsutum* L.

Keywords: Herbicide-resistant crops, transgenic crops.

INTRODUCTION

Glyphosate-resistant (GR) cotton is widely used throughout the United States. Cotton cultivars containing a herbicide resistance gene accounted for 78 and 61% of all cotton planted in North Carolina and the United States, respectively, in 2005 (Anonymous 2005a). Glyphosate-resistant systems have become extremely popular with cotton producers for several reasons. Glyphosate provides broad-spectrum weed control with limited environmental impact. Glyphosate-resistant cotton systems allow for reductions in the use of soil-applied residual herbicides, which consequently reduces the potential for these herbicides to leach into groundwater (Blanchard and Donald 1997; Buhler et al. 1993; Pantone et al. 1992; Wilcut et al. 1995). Glyphosate is degraded by soil microbes and tightly adsorbed to soil colloids, which accounts for its lack of soil activity (Duke 1988; Sprankle et al. 1975a, 1975b). In addition, many GR cotton cultivars also contain

transgenic *Bacillus thuringiensis* insect technology, thus enhancing the utility of GR technology.

In addition to the positive environmental aspects of glyphosate, grower acceptance is due to the low cost and the simplicity of the GR systems (Shaner 2000). Since glyphosate is a broad-spectrum herbicide and has flexibility of application timing in glyphosate-resistant event 1[1] (GRE1) cotton [postemergence over the top (POST) up to 4-lf and postemergence-directed from 4-lf through LAYBY], the use of GR cotton increases the flexibility of POST cotton weed management decisions (Anonymous 2005b). However, several researchers have reported abscission of first position bolls (Jones and Snipes 1999; Viator et al. 2003, 2004), which in some years resulted in delayed maturity. Depending on year, Viator et al. (2004) reported an 8% yield reduction following applications of glyphosate POST at 0.84 kg ae/ha followed by glyphosate postemergence-directed at 0.84 kg/ha compared to treatments that did not include glyphosate. Using ^{14}C-glyphosate, enzyme-linked immunosorbent assays, and measurement of shikimic acid accumulation (which accumulates following inhibition of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) [EC 2.5.1.19]), researchers have found increased glyphosate sensitivity in male reproductive tissues (Pline et al. 2002a, 2002b; Viator et al. 2003). Further research indicated that pollen viability as well as increased stigma length reduced pollination and consequently reduced seed cotton yields (Pline et al. 2002b). The lack of glyphosate tolerance in reproductive tissues of GRE1 lead to the development of a new generation of GR cotton.

The new generation, glyphosate-resistant event 2[2] (GRE2) cotton, uses an identical resistance gene but different promoters as compared to the original resistance technology

(Anonymous 2005c). The use of alternate promoters has increased the tolerance in reproductive portions of the plant while maintaining tolerance levels in vegetative parts (Anonymous 2005c). Consequently, glyphosate can be applied POST up to the 14-lf growth stage. Multiple researchers have investigated the physiological behavior of ^{14}C-glyphosate in GRE1 cotton (Pline et al. 2001, 2002a; Viator et al. 2003), however limited research investigating similar responses to glyphosate in GRE2 cotton has been reported.

Previous photoassimilate movement research in cotton used radiolabeled substrates including CO_2 and sucrose (Ashley 1972; Benedict and Kohel 1975; Benedict et al. 1973; Horrocks et al. 1978). However, these substrates are metabolized into multiple plant products, which may complicate analysis and data interpretation. Glyphosate, which is not readily metabolized in plants (Duke 1988; Sandberg et al. 1980) and shares similar translocation patterns to sucrose (Dewey and Appleby 1983; Tardif and Leroux 1993), may offer an additional tool in photoassimilate research. Glyphosate-resistant event 2 cotton in combination with glyphosate may provide tools to study photoassimilate translocation in cotton in the absence of glyphosate toxicity (Feng and Chiu 2005). Even though several researchers observed similar glyphosate and sucrose translocation patterns in a number of weed species (Dewey and Appleby 1983; Shieh et al. 1993; Tardif and Leroux 1993), a comparison of glyphosate and sucrose translocation in GR cotton or other GR crops has not been conducted.

Due to the commercial availability of two GR events in cotton and properties of glyphosate translocation, our objectives were (1) to evaluate absorption and translocation of glyphosate in these two events at different cotton growth stages and (2) to compare

glyphosate and sucrose translocation patterns in GRE2 cotton as influenced by cotton growth stage.

MATERIALS AND METHODS

Plant Material. Deltapine '5415 RR' (GRE1) and an experimental GRE2[2] variety of cotton were planted in 12-cm pots containing a commercial potting medium[3] and grown in a polyethylene covered greenhouse maintained at 28 $\pm$ 2 C constant temperature where natural sunlight was supplemented 4-h daily with mercury halide lights, providing a 16-h day length. For the comparison of glyphosate and sucrose, a GRE2 cotton variety was planted in 30-cm pots and grown at similar environmental conditions described for the previous experiment. Studies for GR event and glyphosate/sucrose comparisons were conducted from August 2003 to February 2004 and from August 2005 to December 2005, respectively.

^{14}C-Glyphosate Treatments and Sampling for Glyphosate-Resistant Event Comparison. Both cotton events were broadcast-treated at the 4-lf and 8-lf growth stage with glyphosate[4] POST at 0.84 kg/ha. Before each POST glyphosate treatment, a 20-cm^2 area of the fourth and eighth true leaf was covered with aluminum foil to protect spray from coming in contact with the leaf surface. Immediately after broadcast application of glyphosate, aluminum foil was removed and 10 1- ? l droplets of ^{14}C-glyphosate[5] containing 4.2 kBq plus 0.25% non-ionic surfactant[6] were manually applied with a microsyringe to each previously protected leaf portion (Pline et al. 2001).

Plants were harvested at 1, 3, 5, and 7 d after treatment (DAT). Absorption was determined by rinsing the 20-cm^2 treated portion of the leaf with 10 ml of a

methanol:water (1:1, v/v) mixture plus 0.25% non-ionic surfactant[6] (Askew and Wilcut 2002). A 1.0-ml aliquot was taken from the leaf rinsate, diluted in 15 ml scintillation fluid[7] and radioactivity was quantified with liquid scintillation spectrometry (LSS)[8]. All plants were then divided into 1) treated leaf, 2) leaves above treated leaf, 3) leaves below treated leaf, 4) stems below treated leaf, and 5) roots. The treated leaf was removed at the point of attachment to the stem, which also determined the division for above and below treated-leaf sections. If present, the developing sympodia were removed and placed in separate bags. Plant parts were dried for 48 h at 50 C for 2 d, weighed, homogenized, and combusted with a biological sample oxidizer[9]. Radioactivity in the oxidized samples was quantified by LSS.

^{14}C-Glyphosate Treatments and Sampling for Glyphosate/Sucrose Comparison. Ten1-? l droplets of either ^{14}C-glyphosate[5] or ^{14}C-sucrose[10] containing 5 kBq plus non-ionic surfactant[5] at 0.25% (v/v) were added to each plant (Pline et al. 2001; Tardif and Leroux 1993). Radiolabel compounds were applied to the upper most fully expanded leaf (fourth mainstem leaf from the apex) of cotton plants at the 5- and 10-lf growth stages.

Plants were harvested at 7 and 14 DAT. Plants treated at the 5-lf growth stage were divided into: 1) treated leaf, 2) leaves above treated leaf, 3) stems above treated leaf, 4) sympodial tissue (leaves, stems, and fruits) above treated leaf, 5) leaves below treated leaf, 6) stems below treated leaf, 7) sympodial tissue (leaves, stems, and fruits) below treated leaf, and 8) roots. Plants treated at the 10-lf growth stage were divided into: 1) treated leaf, 2) leaves above treated leaf, 3) stems above treated leaf, 4) leaves below treated leaf, 5) stems below treated leaf, 6) sympodial leaves above treated leaf, 7) sympodial stems above treated leaf, 8) sympodial fruits above treated leaf, 9) sympodial leaves below

treated leaf, 10) sympodial stems below treated leaf, 11) sympodial fruits below treated leaf, and 12) roots. Radioactivity quantification for absorption and translocation was conducted as previously described.

Experimental Design and Data Analysis. Studies were arranged in split blocks with randomization within blocks. The blocks in GR events studies were composed of the two varieties with the four harvest timings randomized within varieties while blocks in glyphosate/sucrose comparison were composed of the two cotton growth stages with the four harvest timings randomized within ^{14}C compound. Glyphosate-resistant event and glyphosate/sucrose studies contained four and three replications of treatments, respectively. All experiments were repeated in time. All data were subjected to ANOVA using general linear and mixed models in SAS[11] for glyphosate resistance event and glyphosate/sucrose comparisons, respectively. Results were averaged over trials due to a lack of significant trial main effect. Data were analyzed for main effects and interactions. Mean separations were performed on data using Fisher's Protected LSD at P=0.05. When interactions were significant, LSD (0.05) tests were performed separately across the levels of a given factor within the levels of the other factor.

RESULTS AND DISCUSSION

^{14}C-Glyphosate Absorption for Glyphosate-Resistant Event Comparison. Absorption increased from 18% of applied ^{14}C-glyphosate at 1 DAT to 36% at 7 DAT in GRE2 cotton (Figure 1). Absorption increased at similar rates in GRE1 cotton at the 4-lf growth stage (9 and 29% at 1 and 7 DAT, respectively). Even though differences in absorption were

observed between GR events at the 4-lf growth stage, the overall response was similar, indicating that the biological significance of these differences is probably minimal. In 8-lf cotton, absorption was not different between GR events. Absorption increased from 14% at 1 DAT to 33% at 7 DAT. Pline et al. (2001) reported 19, 29, 45, and 41% absorption of applied ^{14}C-glyphosate at 4-lf, 8-lf, 12-lf, and midbloom stages of GRE1, respectively, averaged over application method (mature leaf and stem) and harvest timing (3 and 7 DAT). At 100% relative humidity, non-transgenic cotton grown at 22 and 32 C absorbed 36 and 43% of the applied ^{14}C-glyphosate, respectively, with the inclusion of a surfactant (Wills 1978). Differences in amounts of absorbed ^{14}C-glyphosate may reflect differences in environmental conditions such as relative humidity and temperature (Masiunas and Weller 1988; Reddy 2000; Wills 1978).

^{14}C-Glyphosate Translocation for Glyphosate-Resistant Event Comparison. Translocation of ^{14}C-glyphosate in cotton was not influenced by trials, events, or harvest timings, thus data were averaged over trials, events, and harvest timings (Table 1). The greatest amount of radioactivity remained in the treated leaf, regardless of cotton growth stage. Since ^{14}C-glyphosate generally follows a source-to-sink translocation pattern (Dewey and Appleby 1983; Sandberg et al. 1980, Tardif and Leroux 1993), the leaf that received the radiolabel was still expanding, potentially reducing further export into other active metabolic sinks. In addition, limited glyphosate export has been reported due to surfactant damage from manual droplet applications of glyphosate in velvetleaf (*Abutilon theophrasti* Medikus) (Feng et al. 1998, Ryerse et al. 2004). When glyphosate was applied to young leaves near the apex of non-transgenic cotton, Wills (1978) also showed limited translocation from these expanding leaves.

Even though the greatest amount of absorbed ^{14}C remained in the treated leaf, significant levels of translocation were observed at both cotton growth stages (Table 1). In 4-lf cotton, the tissue below the treated leaf accumulated similar amounts of ^{14}C-glyphosate, ranging from 10 to 15%. Leaves above the treated leaf were also significant sinks for ^{14}C-glyphosate, equivalent to stems below the treated leaf and roots. Glyphosate translocation in 8-lf cotton also followed a source-to sink relationship. However, the leaves below the treated leaf accumulated 7.2% of the absorbed glyphosate, the greatest level of accumulation compared to other plant parts. The leaves above the treated leaf and roots were active sinks, but accumulated less ^{14}C-glyphosate than the tissues below the treated leaf.

Comparing 4- and 8-lf cotton, more ^{14}C-glyphosate was retained in the treated leaf of 8-lf cotton, which consequently limited translocation to other plant parts. GRE1 cotton treated with glyphosate POST at 4- and 8-lf stages retained 10 and 27% of the applied ^{14}C (Pline et al. 2001). The differences in ^{14}C-glyphosate retention in the treated leaf may explain the observed differences in glyphosate accumulation in plant parts between the two cotton growth stages.

Absorption for Glyphosate/Sucrose Comparison. Absorption of ^{14}C compounds (glyphosate and sucrose) by GRE2 cotton was not influenced by trial, ^{14}C compound, or growth stage (data not shown). Thus data were averaged over trials, ^{14}C compounds, and growth stages. Cotton absorbed 36 and 28% of the applied dose of ^{14}C compounds at 7 and 14 d after treatment, regardless of growth stage and ^{14}C compound (data not shown).

Translocation for glyphosate/sucrose comparison. Translocation of ^{14}C-compounds in GRE2 cotton was not influenced by trials or harvest timings, thus data were averaged over

trials and harvest timings (Table 2). The greatest amount of ^{14}C remained in the treated leaf, regardless of growth stage or compound. However, more ^{14}C-sucrose was translocated from the treated leaf compared to ^{14}C-glyphosate at both cotton growth stages (Table 2). McAllister and Haderlie (1985) also reported high retention (> 70% of absorbed) of $^{14}CO_2$ and ^{14}C-glyphosate in Canada thistle [*Cirsium arvense* (L.) Scop.].

Due to these differences in ^{14}C export from the treated leaf (Table 2), the quantities of ^{14}C in all tissues except the treated leaf were summed and a percentage of exported ^{14}C was calculated for each plant part. After accounting for differences in ^{14}C export, no translocation differences between glyphosate and sucrose were observed, regardless of growth stage. Thus, data were shown averaged over ^{14}C compounds (Table 3).

In five-leaf cotton, 35 and 11% of the translocated ^{14}C were exported out of the treated leaf to apical leaves and roots, respectively (Table 3). These observed translocation patterns were similar to previous research using either ^{14}C-glyphosate or ^{14}C-sucrose (Dewey and Appleby 1983; Sandberg et al. 1980; Shieh et al. 1993; Tardif and Leroux 1993). The leaves and stems below the treated leaf accumulated 19 and 18%, respectively, of the translocated radioactivity (Table 3). Even though tissues below the treated leaf were not meristematic regions at this phenological stage, the accumulation may have been due to continued tissue expansion in both leaves and stems. The levels of radioactivity in stems below the treated leaf may also be explained by the transport mechanisms contained in the stems. Similarly when glyphosate was applied POST to 4-lf cotton, Pline et al. (2001) reported a glyphosate concentration of 256, 127, 57, and 53 Bq/g dry tissue in immature leaves and buds, stems, mature leaves, and roots, respectively. Since roots are a major sink for glyphosate and sucrose (Dewey and Appleby 1983; McAllister and Haderlie 1985;

Sandberg et al. 1980; Shieh et al. 1993; Tardif and Leroux 1993), the stems between the source and sink were more likely to contain larger amounts of radioactivity due to the transport mechanisms in the stem.

Even though translocation patterns were different between cotton growth stages, the distribution continued to follow a source-to-sink relationship. Previous research has also shown that ^{14}C-glyphosate translocation patterns were influenced by cotton growth stage (Pline et al. 2001). Cotton at the 10-leaf growth stage usually contains multiple metabolic sinks, including shoot and root apical meristems as well as the expanding tissue of multiple fruiting branches, which include leaves, stems, and fruits (Mauney 1986). The mainstem leaves and stems below the treated leaf contained 24 and 23% of the translocated radioactivity, respectively (Table 3). In 10-leaf cotton, the expanding sympodial tissues below the source leaf contained 22% of the translocated activity. The majority of the activity in sympodial tissue below the source leaf was contained in the leaves probably due to the chronology of tissue development, where the leaf, stem, and fruit expand in successive order within each position of the sympodia (Mauney 1986). The expanding leaves above the source leaf and roots continued to be metabolic sinks, receiving 11 and 9% of the translocated activity, respectively (Table 3). The sympodial tissues above the source leaf also received some activity (6% of translocated), however, these sinks may not be as great compared to more developed sympodial tissue below the source leaf (Mauney 1986). Based on a compilation of research data on carbohydrate distribution in bolls, Schubert et al. (1986) concluded that boll size and age where significant factors in photoassimilate accumulation. In our study, the most mature sympodial tissues were below the treated leaf, which may explain the greater partitioning into this region.

Since cotton is a perennial crop grown as an annual, the number and strength of sink tissue varies by developmental stage of growth. In vegetative stages, the shoot and root apical meristems are the primary sink tissues, which is evidenced by the level of ^{14}C translocated to these tissues (35 and 11% of the translocated ^{14}C, respectively). When cotton begins reproductive growth, multiple sink tissues arise including sympodial branches (leaves, stems, and fruits) on numerous nodes. Due to the increase in sink number, translocation patterns are altered. For examples, translocation of ^{14}C-compounds increased by 5 and 11 percentage points in stems and sympodial leaves below the treated leaf, respectively, comparing 5- and 10-lf cotton. In addition, the expanding leaves above the treated leaf received numerically less radioactivity, potentially indicating a reduction in sink strength.

Tardif and Leroux (1993) showed that translocation of glyphosate and sucrose were similar in 3 of 5 evaluated quackgrass [*Elytrigia repens* ((L.) Beauv.] biotypes. Correlations for ^{14}C- glyphosate and ^{3}H-sucrose localization in tips, buds, and rhizome segments of quackgrass were 0.89, 0.93, and 0.98, respectively (Shieh et al. 1993). In a field experiment, McAllister and Haderlie (1985) showed similar translocation patterns between metabolized $^{14}CO_2$ and ^{14}C-glyphosate in Canada thistle, depending on experimental environment. Additional data indicated differences in shoot/root partitioning of glyphosate and sucrose where glyphosate preferentially accumulated in roots and shoots of Canada thistle and tall morningglory [*Ipomoea purpurea* (L.) Roth.], respectively (McAllister and Haderlie 1985; Dewey and Appleby 1983). Comparing ^{14}C-sucrose and ^{14}C-glyphosate, our data did not exhibit different translocation patterns to specific tissues,

possibly due to the greater number of sinks in reproductive cotton compared to Canada thistle and tall morningglory.

These data show that glyphosate is translocated to metabolically active tissues like shoot and root apical meristems, regardless of GR cotton event. The evaluation of physiological behavior of glyphosate and sucrose showed equivalent absorption, regardless of cotton growth stage. However, transport of glyphosate from the treated leaf was less than sucrose at both growth stages. Due to these differences in transport from the treated leaves, an evaluation of translocated ^{14}C from the treated leaf showed no differences between glyphosate and sucrose. Translocation patterns varied with cotton growth stages probably due to the increase in potential metabolic sinks as cotton matures (Mauney 1986). Based on these data and previous research (McAllister and Haderlie 1985; Shieh et al. 1985; Tardif and Leroux 1993), glyphosate may have potential for use as a model system to study photoassimilate movement in GR cotton.

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Table 1. Translocation of ^{14}C-glyphosate in cotton. Translocation values are a percentage of absorbed 14C-glyphosate, averaged over glyphosate-resistant event and harvest timing.

Plant part	Four-leaf stage	Eight-leaf stage	
	Translocation		
	% of absorbed ^{14}C		LSD (0.05)[b]
Leaves above treated leaf	6.2	2.7	NS
Treated leaf	57.3	82.3	24.0
Leaves below treated leaf	15.4	7.2	7.3
Stems below treated leaf	11.7	5.1	NS
Roots	9.5	2.1	NS
Reproductive shoots	NA	0.6	NA
LSD (0.05)[a]	7.4	1.4	

[a] Fisher's Protected LSD test at P=0.05 calculated for values within columns.

[b] Fisher's Protected LSD test at P=0.05 calculated for values within rows.

Table 2. Translocation of glyphosate and sucrose in 5- and 10-leaf cotton as a percentage of absorbed ^{14}C, averaged over runs and harvest timings.

Compound	Plant part[a]	Cotton growth stage[b]				
		Five-leaf		10-leaf		LSD[c]
		— % —		— % —		
Glyphosate	ATL – leaves	4.79	de	2.83	e	NS
Glyphosate	ATL – stems	0.81	ef	0.95	e	NS
Glyphosate	ATL – sympodial leaves[d]	0.82	ef	0.73	e	NS
Glyphosate	ATL – sympodial stems	NA		0.31	e	NA
Glyphosate	ATL – sympodial fruits	NA		0.50	e	NA
Glyphosate	Treated leaf	86.13	a	70.40	a	NS
Glyphosate	BTL – leaves	3.39	def	12.87	cd	NS
Glyphosate	BTL – stems	2.10	ef	5.31	de	2.9
Glyphosate	BTL – sympodial leaves[d]	0.47	f	2.67	e	1.2
Glyphosate	BTL – sympodial stems	NA		1.27	e	NA
Glyphosate	BTL – sympodial fruits	NA		0.41	e	NA
Glyphosate	Roots	1.49	ef	1.76	e	NS
Sucrose	ATL – leaves	10.33	c	9.04	de	NS
Sucrose	ATL – stems	1.96	ef	5.14	de	NS
Sucrose	ATL – sympodial leaves[d]	1.58	ef	1.60	e	NS
Sucrose	ATL – sympodial stems	NA		0.71	e	NA
Sucrose	ATL – sympodial fruits	NA		1.03	e	NA
Sucrose	Treated leaf	70.41	b	23.76	b	15.1
Sucrose	BTL – leaves	4.82	de	12.51	cd	3.7
Sucrose	BTL – stems	6.66	cd	18.64	bc	NS
Sucrose	BTL – sympodial stems[d]	0.97	ef	12.34	cd	NS
Sucrose	BTL – sympodial fruits	NA		6.66	de	NA
Sucrose	BTL – sympodial leaves	NA		1.18	e	NA

Table 2 (continued)

Sucrose	Roots	3.01	def	7.40	de	NS

[a] Abbreviations: ATL, above treated leaf; BTL, below treated leaf.

[b] Means within a column followed by the same letter are not different according to Fisher's Protected LSD test at P=0.05.

[c] Fisher's Protected LSD test at P=0.05 for data within columns.

[d] Percent of radioactivity in the part labeled sympodial leaves includes all sympodial tissues including leaves, stems, and fruits in 5-lf cotton.

Table 3. Translocation of ^{14}C compounds (glyphosate and sucrose) in 5- and 10-leaf cotton as a percentage of exported ^{14}C from the treated leaf, averaged over runs and harvest timings.[a]

Plant part[b]	Cotton growth stage[c]				LSD[d]
	Five-leaf		10-leaf		
	——— % ———		——— % ———		
ATL – leaves	35.20	a	11.33	bc	NS
ATL – stems	6.41	d	5.17	de	NS
ATL – sympodial leaves[e]	6.12	d	2.65	e	NS
ATL – sympodial stems	NA		1.07	e	NA
ATL – sympodial fruits	NA		1.78	e	NA
BTL – leaves	19.46	b	23.85	a	NS
BTL – stems	18.11	b	23.37	a	1.9
BTL – sympodial leaves[e]	3.23	d	13.69	b	2.0
BTL – sympodial stems	NA		6.81	cde	NA
BTL – sympodial fruits	NA		1.55	e	NA
Roots	10.90	c	8.74	bcd	NS

[a] Translocation is calculated based on the amounts of radioactivity transported out of the treated leaf.

[b] Abbreviations: ATL, above treated leaf; BTL, below treated leaf.

[c] Means within a column followed by the same letter are not different according to Fisher's Protected LSD test at P=0.05.

[d] Fisher's Protected LSD test at P=0.05 for data within columns.

[e] Percent of radioactivity in the part labeled sympodial leaves includes all sympodial tissues including leaves, stems, and fruits in 5-lf cotton.

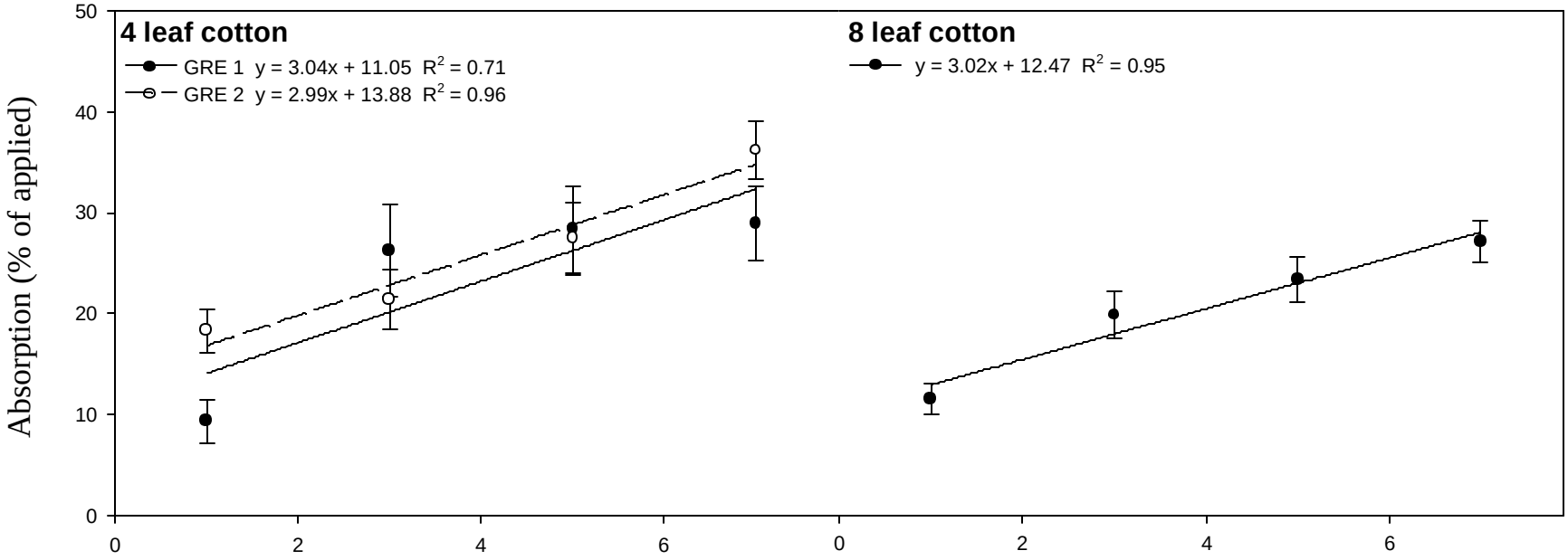

Figure 1. Absorption of glyphosate in 4-lf and 8-lf cotton is shown as a percentage of applied, averaged trials. In 8-lf cotton, absorption is also averaged over trials and glyphosate resistance events.

Translocation of spot-applied glyphosate as influenced by mepiquat chloride in cotton.

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Abstract: Studies examined various morphological characteristics and ^{14}C-glyphosate translocation in cotton as influenced by ^{14}C-treatment timing [0, 7, 14, and 21 d after mepiquat chloride (MC) treatment (DMC)] and cotton plant growth regulator regime (none and MC at 49 g ai ha^{-1}). Due to the large number of plant parts, data for a five-node section of each plant was recorded for each parameter (the mainstem leaf on node B received the ^{14}C-glyphosate treatment). No significant differences in plant height, leaf area, and specific leaf weight were observed for any ^{14}C-glyphosate timing or MC combination. At the time of MC treatment and 21 DMC, total fruit retention was 85 and 70%, respectively. Even though all plants retained at least 86% of all first position fruits, a significant decline was observed from the day of MC treatment to 21 DMC (92 and 86%, respectively). Fresh and dry weight data showed similar responses with multiple first position fruits of MC-treated plants accumulating more biomass compared to first position fruits of non-treated plants. For dry weight of plants parts, first position fruits on nodes A, B, and C of MC-treated plants accumulated 30, 35, and 45% greater biomass, respectively, compared to first position fruits of non-treated plants. Absorption of ^{14}C-glyphosate was not influenced by either ^{14}C-glyphosate timing or MC treatment. Even though no significant observations were found on node A, several numerical observations offer support for reduced leaf expansion and increased biomass accumulation in response to MC treatment. In node A, second position fruits on MC-treated plants contained 68% more ^{14}C-glyphosate g^{-1} of dry tissue than second position fruits of non-treated plants. In addition, numerical increases in ^{14}C-glyphosate concentration in the leaf and stem of the second position of node A in MC-treated plants were observed compared to the same parts of non-treated plants. On node D, first position fruits of MC-treated plant contained nearly

6 and 7 times the ^{14}C-glyphosate concentration at 0 and 21 DMC treatment compared to the respective timing for non-treated plants. In addition, all other comparisons with first position fruit were numerically greater with MC treatment. These data support previous research that showed increased fruit weight and provides insight into the potential for MC treatment to alter the source-to-sink relationship in reproductive cotton.

Nomenclature: Glyphosate, mepiquat chloride, cotton, *Gossypium hirsutum* L.

Key words: Photoassimilates, plant growth regulators.

Introduction

Development and retention of cotton fruits are influenced by supply and demand of plant photoassimilates. The complex balance of these source-to-sink relationships varies by plant position and age (Ashley 1972; Benedict and Kohel 1975) as well as environmental conditions (Guinn 1982). Multiple researchers have investigated photoassimilate patterns in cotton with $^{14}CO_2$ (Ashley1972; Benedict and Kohel 1975; Horrocks et al. 1978) and ^{14}C-glyphosate (Feng and Chiu 2005). Ashley (1972) and Horrocks et al. (1978) showed that the subtending leaf of a fruit was the primary photoassimilate source. Furthermore, Ashley (1972) showed that at least 88% of the ^{14}C-photoassimilate remained in the treated sympodial branch. With different leaf morphology of normal and superokra leaf cotton, Horrocks et al. (1978) revealed that the smaller superokra leaves supplied 88% of the photoassimilate to the subtending fruits compared to normal leaves, indicating that photoassimilate translocation patterns were similar regardless of leaf morphological differences.

Glyphosate, a commonly used systemic herbicide, is symplastic in nature and is translocated in the phloem following a source-to-sink relationship (Dewey and Appleby 1983; Sandberg et al. 1980; Tardif and Leroux 1993). Due to these properties, similar patterns of glyphosate and sucrose translocation have been reported in Canada thistle [*Cirsium arvense* (L.) Scop.], cotton, quackgrass [*Elytrigia repens* (L.) Nevski], and tall morningglory [*Ipomoea purpurea* (L.) Roth.] (Dewey and Appleby 1983; Harker and Dekker 1988; Klevorn and Wyse 1984; McAllister and Haderlie 1985; Shieh et al. 1993; Tardif and Leroux 1993; Thomas et al. 2006). After accounting for differences in export between glyphosate and sucrose, Thomas et al. (2006) showed that glyphosate and sucrose were distributed equally, regardless of cotton growth stage (5- and 10-leaf). Furthermore, Tadrif and Leroux (1993) found that glyphosate and sucrose translocation was not significantly different in three biotypes of quackgrass. Correlations for ^{14}C- glyphosate and ^{3}H-sucrose localization in tips, buds, and rhizome segments of quackgrass were 0.89, 0.93, and 0.98, respectively (Shieh et al. 1993). However, Harker and Dekker (1988) have shown that glyphosate translocation is less than sucrose translocation, but follows a similar translocation pattern. Other research has found similar translocation relationships between photoassimilates and glyphosate, depending on plant species, phenological stage, and environmental stress levels (Dewey and Appleby 1983; Klevorn and Wyse 1984; McAllister and Haderlie 1985). However, glyphosate treatment in susceptible species may limit its own translocation (Geiger and Bestman 1990; Hetherington et al. 1999). In order to utilize glyphosate as a photoassimilate model, genetically engineered glyphosate-resistant plants may be used to reduce limitation of glyphosate translocation such as in glyphosate-susceptible cotton (Feng and Chiu 2005; Hetherington et al. 1999). A new

generation of glyphosate-resistant cotton (GR2) allowed glyphosate application up to seven days before harvest, due to reported increased reproductive tolerance. By combining the herbicidal characteristics of glyphosate and glyphosate-resistant technology in cotton, Feng and Chiu (2005) examined glyphosate translocation as influenced by plant position to show similar patterns of translocation from sympodial leaves to adjacent fruits.

Many cotton production systems include the use of plant growth regulators (PGRs) to manage vegetative cotton growth. Mepiquat chloride and mepiquat pentaborate, two onium-type growth regulators commonly used in these systems, inhibit gibberillic acid synthesis by stopping the conversion of geranylgeranyl diphosphate to *ent*-kaurene, consequently reducing cell enlargement and the rate of cell division (Rademacher 2000; Srivastava 1993). The visual effects of these PGRs include reduced stem and leaf expansion and cotton reaching maturity earlier than cotton not treated with PGRs (Reddy et al. 1990, 1996; York 1983a, 1983b). However, cotton yield responses were variable (Kerby 1985). Since PGRs have been shown to alter cotton canopy architecture and fruit maturity, photoassimilate translocation patterns are presumably altered by these applications. Based on increased dry matter partitioning into fruits, Zhao and Oosterhuis (2000) postulated that these observed differences may be due to altered photoassimilate translocation. However, Zhao and Oosterhuis (2000) did not observe any translocation differences of metabolized $^{14}CO_2$ from the leaf into the adjacent fruit. Even though research has not confirmed differences in photoassimilate translocation in response to MC treatment using $^{14}CO_2$ and ^{14}C-sucrose technology, glyphosate and glyphosate-resistant technology offer new tools for investigating photoassimilate translocation patterns in cotton (Feng and Chiu 2005).

Since glyphosate movement has been correlated with sucrose movement in cotton (Thomas et al. 2006) and multiple weed species (Dewey and Appleby 1983; Harker and Dekker 1988; Klevorn and Wyse 1984; McAllister and Haderlie 1985; Shieh et al. 1993; Tardif and Leroux 1993), our objectives were to evaluate translocation of glyphosate at different timings relative to a single MC treatment.

Materials and Methods

Plant material. An experimental GR2 cotton variety was planted in 30-cm pots (9 liter volume) containing a commercial potting medium[1] and grown in a polyethylene covered greenhouse maintained at 28 $\pm$ 2 C constant temperature where natural sunlight was supplemented 4 h daily with mercury halide lights, providing a 16-h day length. After the 8-lf stage, all plants received 300 mL of fertilizer solution[2] (1.3 cm^2/L) weekly.

^{14}C-Glyphosate Treatments and Sampling. A two-factor factorial arrangement of treatments experiment was conducted with the first factor consisting of PGR regimes of no PGR or MC[2] at 49 g ai ha^{-1} applied at first bloom (plants with 14 nodes). Time of ^{14}C-glyphosate[4] spot application on 0, 7, 14, and 21 DMC application was the second factor. ^{14}C-glyphosate at 8,333 Bq plus non-ionic surfactant[5] at 0.25% (v/v) per plant was manually applied in 10 1-uL droplets on the upper most fully expanded leaf of each plant (Pline et al. 2001).

Prior to destructive harvests, total plant height was measured and fruit retention was mapped for each plant. All plants were harvested at 14 d after each ^{14}C-glyphosate timing and were partitioned into nodes, positions within each sympodial branch, and parts within each position. Each mainstem nodal section contained a leaf and stem section while each

sympodial position contained a leaf, stem, and fruit (square/boll). Fresh weight, dry weight, and leaf area (where applicable) were recorded for each part. Absorption was determined by rinsing the treated portion of the leaf with 10 ml of a methanol:water (1:1 v/v) plus a non-ionic surfactant[5] (0.25% v/v). A 1.0-ml aliquot was taken from the leaf rinsate, diluted in 15 ml scintillation fluid[6] and radioactivity was quantified with liquid scintillation spectrometry (LSS)[7]. Divided plant parts were dried for 48 h at 70 C, weighed, and combusted with a biological sample oxidizer[8]. Radioactivity in the oxidized samples was quantified by LSS. Due to the large number of samples, only a five-node section of each plant was processed for ^{14}C quantification. The selected regions included the treated leaf node (node B), one node below the treated leaf (node A), and three consecutive nodes above the treated leaf (nodes C, D, and E). ^{14}C-glyphosate was applied to the mainstem leaf on nodes 11 to 14, depending on ^{14}C-application timing (Table 1). All plant parts for the first replication were oxidized to estimate the total recovery. Based on this estimation, at least 80% of the absorbed ^{14}C-glyphosate was found in this five-node section.

Experimental Design and Data Analysis. Studies were arranged in randomized complete blocks with four replications. Studies were repeated once in time. Data were tested for homogeneity of variance by plotting residuals. ^{14}C-glyphosate concentration (Bq g^{-1} dry tissue) was log transformed to improve homogeneity of variance. Homogeneity of other parameters was not improved with transformations. Analysis of variance conducted using a mixed model in SAS[9] revealed no trial by treatment interaction, thus data were averaged over trials. Data were analyzed for main effects and interactions. Mean separations were performed on data using LS means at P=0.05.

Results and Discussion

Cotton morphology. No significant differences in plant height were observed for any ^{14}C-glyphosate timing or PGR combination (Table 1). Previous research has shown height reduction with MC under variable environmental conditions (Pettigrew and Johnson 2005; Nichols et al. 2003; Reddy et al. 1996; Zhao and Oosterhuis 2000). The lack of cotton height reductions in this experiment may be due to the MC application timing and environmental conditions. Mepiquat chloride was applied at 49 g ha^{-1} at first bloom, which may not provide adequate growth regulation in optimum greenhouse conditions. Previous research has shown that multiple applications are needed in some environmental situations to adequately limit vegetative growth (Edmisten 1994, 2004; Hake et al. 1991; Watkins et al. 1998). Since our objective of this study was to evaluate ^{14}C-glyphosate translocation patterns at various time intervals following MC treatment, multiple MC treatments were not possible.

Total fruit retention and first position fruit retention were not influenced by trial or MC treatment, thus data were averaged over trials and MC treatment (Table 2). Total fruit retention and first position fruit retention declined as cotton matured. On the d of MC treatment and 21 DMC, total fruit retention was 85 and 70%, respectively. Even though all plants retained at least 86% of all first position fruits, a significant decline was observed from the day of MC treatment to 21 DMC treatment (92 and 86%, respectively). Fruit abortion is commonly observed near the end of the fruiting cycle (Guinn 1982). Fruit abortion is a normal biological response to a number of interrelated factors, including light, temperature, water stress, insects, disease, and available nutrients (Guinn 1982).

Furthermore, the level of retention is based on the balance between the number of developing fruits and leaf area of healthy leaves (Hake et al. 1991).

Even though our data did not show significant differences in fruit retention or placement on the plant, previous research has reported greater retention on more basal fruiting branches and lower retention on more apical fruiting branches following MC treatment (Hake et al. 1991; Kerby et al. 1986). The ability of MC to alter fruiting patterns is stage dependent (A.C. York, personal communication). Furthermore, Kerby et al. (1986) reported more harvestable fruits on the first, second, and monopodial positions on nodes 8 to 12 of MC-treated plants. With the use of MC at 49 g ha^{-1}, York (1983a) reported greater fruit production (fruit m^{-2}) at 3 of 6 locations. At population of 235,000 cotton plants ha^{-1}, York (1983b) reported a 14% increase in fruit production with the use of MC at 49 g ha^{-1}. In addition, Cook and Kennedy (2000) reported that MC treatment can increase fruit retention on the second position of lower sympodial branches in response to varying levels of fruit loss. Based on the increased fruit retention in lower portions of the plant, Cook and Kennedy (2000) postulated that this higher retention would maintain sink strength in the lower portion of the plant canopy, which may consequently limit further fruit develop on higher nodes (Kerby et al. 1986).

Leaf area was not influenced by trial, ^{14}C-glyphosate timing, or MC treatment, thus data were averaged over trial, ^{14}C-glyphosate timing, and MC treatment (Table 3). Leaf area decreased with increasing nodal position. The treated leaf [the mainstem leaf subtending nodes 11 to 14, depending on ^{14}C-glyphosate timing (Table 1)] was 177 cm^2. Mainstem leaf area for nodes C, D, and E was 13, 28, and 45% less, respectively, compared to the mainstem leaf subtending node B. Comparing the leaf subtending the first

position of each node, a similar decline in leaf size for fruiting positions was observed. In addition to a smaller leaf size for more vertical nodal positions, leaf size on sympodial branches was also smaller with increasing distance from the mainstem, regardless of node. On node B, the leaf subtending the first and second position was 74 and 22 cm^2, respectively. Sadras (1995) has discussed the implications for reduced leaf area with increasing vertical and horizontal position within the plant canopy on fruit distribution. Mepiquat chloride treatments have been reported to reduce leaf area (Reddy et al. 1990, 1996; Zhao and Oosterhuis 2000). Total leaf area of MC-treated plants was reduced by 16% compared to non-treated plants (Reddy et al. 1996). In different temperature regimes, Reddy et al. (1990) observed that leaf area was reduced the least in the lowest and highest temperature regimes. However, specific leaf weight responses were variable (Reddy et al. 1990; Zhao and Oosterhuis 2000). Our data did not show any difference in specific leaf weight or leaf area in response to MC treatment (data not shown). The timing of MC treatment and environmental conditions may have allowed maximum leaf expansion on the examined nodes before MC treatment.

Fresh weight was not influenced by trial or ^{14}C-glyphosate timing, thus data were averaged over trial and ^{14}C-glyphosate timing (Table 4). Significant interactions between MC treatment and plant parts were observed for node A, C, and D. All other nodes exhibited only a plant part main effect. Fresh weight of first position fruit on MC-treated plants was 25 and 48% greater on nodes A and C, respectively, compared to first position fruits on non-treated plants. On node D, similar numerical differences were observed. Even though leaf area differences were not significant for MC treatment, the mainstem leaf of MC-treated plants weighed 17% less than the mainstem leaf of non-treated plants,

which may be indicative of a reduction in leaf area or a change in carbon allocation patterns.

Dry weight was not influenced by trial or ^{14}C-glyphosate timing, thus data were averaged over trial and ^{14}C-glyphosate timing (Table 5). Significant interactions between MC treatment and plant parts were observed for nodes A, B, C, and D. Similar to fresh weights of plants parts, first position fruits on nodes A, B, and C of MC-treated plants accumulated 30, 35, and 45% greater biomass, respectively, compared to first position fruits of non-treated plants. Conversely, the mainstem leaf of nodes B, C, and D of MC-treated plant accumulated 16, 13, and 16% less biomass, respectively, compared to the corresponding mainstem leaves of non-treated plants. Research has shown significant increases in reproductive biomass production in response to MC treatments (Hake et al. 1991; Kerby et al. 1986; Pettigrew and Johnson 2005; Sawan and Sakr 1990; York 1983, 1983b; Zhao and Oosterhuis 2000). At 5 of 8 locations and averaged over 14 varieties, York (1983a) reported significant fruit weight (g fruit^{-1}) increases ranging from 3 to 10% in response to MC treatment. York (1983b) reported similar increases (3 to 6%) in fruit weight (g fruit^{-1}) at 4 of 5 locations, averaged over nitrogen rates and plant population. A more detailed analysis of fruit composition revealed an increase in seed weight with MC treatments, which accounted for 75% of the increase in fruit weight (York 1983a). Zhao and Oosterhuis (2000) reported similar increases in fruit weight and reduced lint fraction for various MC formulations, depending on year and location. For vegetative parts, Zhao and Oosterhuis showed no differences for dry weight of leaves and petioles (% of total dry weight) between MC formulations and non-treated plants at Carbondale, AR in 1997.

^{14}C-Glyphosate Translocation. Absorption of ^{14}C-glyphosate was not influenced by either ^{14}C-glyphosate timing or MC treatment (Table 1). Absorption of ^{14}C-glyphosate ranged from 89 to 95% of the applied. Pline et al. (2001) showed that 10 to 44% of the applied ^{14}C-glyphosate remained in the leaf wash, depending on the treatment timing and harvest interval. Due to the treatment placement (fourth most apical mainstem leaf), all leaves were near the same phenological stage of development, which limited the variability of ^{14}C-glyphosate absorption associated with difference in leaf age (Feng and Chui 2005).

^{14}C-glyphosate translocation from the source leaf varied by node, position within sympodia, and plant parts within each position (Table 6). For ^{14}C-glyphosate concentration, the plant parts within positions were highly (>0.01) significant at all evaluated nodes. Except for the treated leaf node (node B), the mainstem plant parts generally contained less ^{14}C-glyphosate compared to plant parts on the sympodial branch (Table 7). On nodes C and D, all second position parts contained greater ^{14}C-glyphosate concentrations than any other plant tissue within each node. As shown by previous work (Pline et al. 2001; Sandberg et al. 1980; Viator et al. 2003), ^{14}C-glyphosate is transported to actively growing tissue like the expanding leaf, stem, and fruit of the second position. When ^{14}C-glyphosate was applied to mainstem leaf subtending node 9, 22 and 9% of the applied ^{14}C-glyphosate was exported to the plant and sympodial branch, respectively (Feng and Chiu 2005). However, when glyphosate was applied to the leaf subtending the first position on node 9, similar ^{14}C-glyphosate distribution to the plant and sympodial branch was observed (Feng and Chiu 2005).

A ^{14}C-glyphosate timing by position and plant part interaction was shown for node A ($p=0.1$) and C ($p=0.05$) while a MC treatment by position and plant part interaction was

observed for node A (p=0.1) (Table 6). Several observations offer support for reduced leaf expansion (Reddy et al. 1990, 1996; Zhao and Oosterhuis 2000) and increased biomass accumulation (Hake et al. 1991; Kerby et al. 1986; Pettigrew and Johnson 2005; Sawan and Sakr 1990; York 1983, 1983b; Zhao and Oosterhuis 2000) in response to MC treatment (Table 8). Numerically, the mainstem leaf of non-treated and MC-treated plants accumulated 136 and 38 Bq g^{-1} dry tissue, respectively. In non-treated plants, leaf expansion may have been occurring, which required more photoassimilate compared to non-expanding leaf tissue of MC-treated plants. Second position fruits on MC-treated plants contained 68% more ^{14}C-glyphosate g^{-1} of dry tissue than second position fruits of non-treated plants. In addition, numerical increases in ^{14}C-glyphosate concentration in the leaf and stem of the second position of MC-treated plants were observed compared to the same parts of non-treated plants. Even though the weight of plant parts was not different on the second position, these data indicate that these parts in MC-treated plants were stronger sinks compared to non-treated plants. Futhermore, the larger fruit weight on the first position fruit may have maintained the sink strength in the lower portion of the plant canopy (Cook and Kennedy 2000), allowing for greater ^{14}C-glyphosate translocation into the expanding tissues of the second position of MC-treated plants. Investigations with flurprimidol, another gibberillic acid synthesis inhibitor, on 8-wk old Canada thistle revealed greater translocation of ^{14}C-sucrose (75% of applied) following flurprimidol at 0.54 kg ai ha^{-1} followed by glyphosate at 2.24 kg ai ha^{-1} (7 wk after the flurprimidol treatment) compared to plants receiving only glyphosate (58%) (Tworkoski et al. 1992). Based on these data (Tworkoski 1992), flurprimidol significantly altered the translocation of photoassimilates compared to non-treated plants.

A ^{14}C-glyphosate timing by MC treatment by position and plant part interaction was observed for node C ($p=0.05$) and D ($p=0.01$). MC-treated plants had greater ^{14}C-glyphosate concentration in first position fruits at 0 and 7 DMC on node C and D, respectively, compared to non-treated plants (Table 9). On node D, first position fruits of MC-treated plants contained nearly 6 and 7 times the ^{14}C-glyphosate concentration at 0 and 21 DMC treatment compared to the respective timing for non-treated plants. In addition, all other comparisons with first position fruit were numerically greater with MC treatment. Even though few observations were significant, several trends were apparent. Comparing the stems on the first position of node C, stem of MC-treated plants contained numerically greater ^{14}C-glyphosate concentrations, regardless of ^{14}C-glyphosate timing. This greater concentration of ^{14}C-glyphosate may be indicative of greater translocation to either the adjacent fruit and more distal plant parts, which were active sinks (Table 7).

Even though plant height, leaf area, or specific leaf weights were not significantly influenced by MC treatment, fresh and dry weight of plant parts and ^{14}C-glyphosate translocation were influenced by MC treatment. Plants treated with MC caused increased biomass accumulation in the first position fruits on nodes A, B, and C compared to fruits on non-treated plants. Similar numerical increases were observed for fruit dry weight on node D. Since leaf age was similar among ^{14}C-glyphosate treatments, absorption was not influenced by treatment timing. ^{14}C-glyphosate concentration was influenced by MC treatment (node A) and MC treatment by ^{14}C-glyphosate timing (nodes C and D). Increased ^{14}C-glyphosate concentrations were observed in response to MC treatment in these nodes. These data support previous research that showed increased fruit weight and provides insight into the potential for MC treatment to alter the source-to-sink relationship

in reproductive cotton and utility of using glyphosate to study photoassimilate translocation patterns in GR2 cotton.

Sources of Materials

[1] MetroMix 200, Sun Gro Horticulture, 15831 N.E. 8th Street, Suite 100, Bellevue, WA 98008.

[2] Peters Professional 20-20-20 General Purpose, The Scotts Company, 14111 Scottslawn Road, Marysville, OH 43041.

[3] Mepichlor, Micro Flo Company, P. O. Box 772099, Memphis, TN 38117.

[4] ^{14}C-glyphosate G-8392, Sigma Chemical Co., P. O. Box 14508, St. Louis, MO 63178.

[5] Induce (mixture of alkyl polyoxylkane ether, free fatty acids, and isopropanol), Helena Chemical Co., 5100 Popular Avenue, Memphis, TN 38137.

[6] Scintiverse® SX18-4 Universal Liquid Scintillation Cocktail, Fisher Scientific, 1 Regeant Road, Fair Lawn, NJ 07410.

[7] Packard Tri-Carb 2100TR Liquid Scintillation Spectrometer, Packard Instrument Co., 220 Warrenville Rd., Downers Grove, IL 60515.

[8] Model OX500 Biological Material Oxidizer, R. J. Harvey Instrument Corp., 123 Patterson St., Hillsdale, NJ 07642.

[9] [SAS] Statistical Analysis Systems software, Ver. 8., SAS Institute, Inc., Box 8000, SAS Circle, Cary, NC 27513.

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Table 1. Interaction of ^{14}C-glyphosate timing and mepiquat chloride on the total plant height at harvest, treated leaf node, and absorption of ^{14}C-glyphosate, averaged over trials.

Timing[a] DMC	PGR[b]	Plant height		Treated leaf node		Absorption	
		Mean	SEM[c]	Mean	SEM	Mean	SEM
		cm		Nodal number		% of applied	
0	None	125.4	4.77	11.1	0.23	89.3	2.77
0	MC	130.3	6.48	12.5	0.5	94.1	1.29
7	None	125.1	4.02	12.6	0.42	93.3	1.21
7	MC	130.6	3.14	12.1	0.23	94.0	0.82
14	None	124.3	1.87	11.6	0.37	92.3	1.45
14	MC	126.6	5.00	12.9	0.4	94.6	0.80
21	None	126.8	4.35	12.9	0.55	93.6	0.73
21	MC	129.2	5.69	13.6	0.32	93.7	1.73
LSD (0.05)		NS		0.48		NS	

[a] ^{14}C-glyphosate at 8,333 Bq plus non-ionic surfactant at 0.25% (v/v) per plant was manually applied in ten 1-uL droplets on the upper most fully expanded leaf of each plant. Applications were made at 0 [day of mepiquat chloride (MC) applications], 7, 14, and 21 d after MC treatment (DMC).

[b] PGR regimes were no PGR (none) or MC at 49 g ai ha^{-1} applied at first bloom (MC).

[c] Standard error of the mean.

Table 2. ^{14}C-glyphosate timing main effect on total fruit retention and first position fruit retention, averaged over trials and mepiquat chloride regime.

Timing[a]	Total fruit retention		1st position fruit retention	
DMC	Mean	SEM[c]	Mean	SEM
	%			
0	84.8	4.9	92.1	3.0
7	86.7	4.4	92.8	3.6
14	88.0	3.6	98.7	0.9
21	70.1	5.7	85.9	3.9
LSD (0.05)	13.9		9.5	

[a] ^{14}C-glyphosate at 8,333 Bq plus non-ionic surfactant at 0.25% (v/v) per plant was manually applied in ten 1-uL droplets on the upper most fully expanded leaf of each plant. Applications were made at 0 [day of mepiquat chloride (MC) applications], 7, 14, and 21 d after MC treatment (DMC).

[b] Standard error of the mean.

Table 3. Interaction of nodal position and leaf position within sympodia on leaf area, averaged over trials, ^{14}C-glyphosate timing, and mepiquat chloride.

Position	Leaf area				
	Node A[a]	Node B[b]	Node C	Node D	Node E
	cm^2				
Mainstem	197 a	177 a	155 a	127 a	97 a
First	84 b	74 b	57 b	34 b	13 b
Second	35 c	22 c	4 c	2 c	.

[a] Means within a column followed by the same letter are not different according to LSD test at P=0.05.

[b] All node letters have been arbitrarily defined. Node A is one node below the treated leaf while nodes C, D, and E are one, two, and three nodes above the treated leaf, respectively. Node B is the site of ^{14}C-glyphosate application, with actual nodal values shown in Table 1.

Table 4. Interaction of mepiquat chloride and sympodial position for fresh weight of the various cotton parts within each position on nodes A, C and D, averaged over trials and ^{14}C-glyphosate timing. For nodes B and E, fresh weight is presented by the various cotton parts within sympodial position, averaged over trials, ^{14}C-glyphosate timing, and mepiquat chloride.[a]

PGR[b] regime	Position	Part	Fresh weight									
			Node A[cd]		Node B		Node C		Node D		Node E	
			g									
None	Mainstem	Leaf	5.46	c			4.68	a	3.85	a		
None	Mainstem	Stem	2.34	d			1.26	cde	0.85	cd		
None	First	Leaf	1.92	def			1.54	cd	0.76	cd		
None	First	Stem	0.97	efg			0.65	efg	0.44	ef		
None	First	Fruit	7.80	b			1.72	c	0.88	cd		
None	Second	Leaf	0.76	efg			0.15	g	0.06	gh		
None	Second	Stem	0.33	g			0.12	g	0.04	gh		
None	Second	Fruit	0.72	efg			0.28	g	0.08	gh		
MC	Mainstem	Leaf	5.03	c			4.10	a	3.18	b		
MC	Mainstem	Stem	2.03	de			0.97	def	0.59	def		
MC	First	Leaf	1.96	de			1.24	cde	0.66	cde		
MC	First	Stem	1.03	defg			0.57	fg	0.34	fg		
MC	First	Fruit	10.43	a			3.31	b	0.95	c		
MC	Second	Leaf	0.64	fg			0.17	g	0.04	h		
MC	Second	Stem	0.24	g			0.08	g	0.03	h		
MC	Second	Fruit	0.87	efg			0.18	g	0.08	gh		
	Mainstem	Leaf			4.88	b					2.52	a
	Mainstem	Stem			1.68	c					0.38	b
	First	Leaf			1.78	c					0.25	bc
	First	Stem			0.84	d					0.20	c
	First	Fruit			5.71	a					0.40	b
	Second	Leaf			0.42	d					0.01	d
	Second	Stem			0.19	d					0.01	d
	Second	Fruit			0.56	d					0.02	d

[a] Abbreviation: MC, mepiquat chloride; PGR, plant growth regulator.

[b] PGR regimes were no PGR (none) or MC at 49 g ai ha^{-1} applied at first bloom (MC).

Table 4 (continued)

[c] All node letters have been arbitrarily defined. Node A is one node below the treated leaf while nodes C, D, and E are one, two, and three nodes above the treated leaf, respectively. Node B is the site of ^{14}C-glyphosate application, with actual nodal values shown in Table 1.

[d] Means within a column followed by the same letter are not different according to LSD test at P=0.05.

Table 5. Interaction of mepiquat chloride and sympodial position for dry weight of the various cotton parts within each position on nodes A, B, C, and D, averaged over trials and ^{14}C-glyphosate timing. For node E, dry weight is presented by the various cotton parts within sympodial position, averaged over trials, ^{14}C-glyphosate timing, and mepiquat chloride.[a]

PGR[b] regime	Position	Part	Dry weight									
			Node A[cd]		Node B		Node C		Node D		Node E	
			g									
None	Mainstem	Leaf	1.18	b	1.21	a	1.04	a	0.82	a		
None	Mainstem	Stem	0.67	c	0.50	d	0.32	d	0.16	c		
None	First	Leaf	0.43	cde	0.41	de	0.30	d	0.16	c		
None	First	Stem	0.30	def	0.27	efg	0.17	ef	0.09	d		
None	First	Fruit	1.31	b	0.69	c	0.29	d	0.16	c		
None	Second	Leaf	0.16	ef	0.09	hi	0.04	h	0.01	ef		
None	Second	Stem	0.07	f	0.04	I	0.02	h	0.01	f		
None	Second	Fruit	0.13	f	0.10	ghi	0.05	gh	0.02	ef		
MC	Mainstem	Leaf	1.15	b	1.02	b	0.90	b	0.69	b		
MC	Mainstem	Stem	0.62	c	0.44	de	0.26	def				
MC	First	Leaf	0.44	cd	0.37	def	0.26	de	0.13	cd		
MC	First	Stem	0.32	def	0.23	fgh	0.15	fg	0.08	de		
MC	First	Fruit	1.87	a	1.06	ab	0.53	c	0.16	c		
MC	Second	Leaf	0.14	f	0.09	hi	0.03	h	0.01	f		
MC	Second	Stem	0.06	f	0.04	i	0.01	h	0.00	f		
MC	Second	Fruit	0.13	f	0.10	ghi	0.04	h	0.02	f		
	Mainstem	Leaf									0.49	a
	Mainstem	Stem									0.06	bc
	First	Leaf									0.06	bc
	First	Stem									0.03	cd
	First	Fruit									0.08	b
	Second	Leaf										
	Second	Stem										
	Second	Fruit										

[a] Abbreviation: MC, mepiquat chloride; PGR, plant growth regulator.

[b] PGR regimes were no PGR (none) or MC at 49 g ai ha^{-1} applied at first bloom (MC).

Table 5 (continued)

[c] All node letters have been arbitrarily defined. Node A is one node below the treated leaf while nodes C, D, and E are one, two, and three nodes above the treated leaf, respectively. Node B is the site of ^{14}C-glyphosate application, with actual nodal values shown in Table 1.

[d] Means within a column followed by the same letter are not different according to LSD test at P=0.05.

Table 6. The levels of significant difference for the main effects and their interactions on ^{14}C-glyphosate concentration in cotton for each node.

	Node[a]				
	A	B	C	D	E
^{14}C- timing	NS	NS	NS	NS	NS
PGR regime	NS	NS	NS	NS	NS
^{14}C- timing * PGR regime	NS	NS	NS	NS	NS
position_part[b]	***	***	***	***	***
^{14}C- timing *position_part	*	NS	**	NS	NS
PGR regime *position_part	*	NS	NS	NS	NS
^{14}C- timing * PGR regime *position_part	NS	NS	**	***	NS

[a] *, **, ***, Significantly different at the 0.1, 0.05, and 0.01 probability level, respectively, using a mixed model in SAS.

[b] Prior to analysis, the data representation for each position and parts within each position were merged to simplify data analysis.

Table 7. Interaction of parts within each position and sympodial position for ^{14}C-glyphosate concentration, averaged over trials, ^{14}C-glyphosate timing, and mepiquat chloride.

Position	Part	^{14}C-glyphosate[ab]									
		Node A[c]		Node B		Node C		Node D		Node E	
		Bq g^{-1} dry tissue									
Mainstem	Leaf	87	a	7181	a	55	cd	51	f	45	c
Mainstem	Stem	54	a	189	b	55	c	59	e	106	b
First	Leaf	44	b	82	c	63	cd	204	c	183	a
First	Stem	45	b	86	c	50	d	123	d	174	a
First	Fruit	52	a	121	b	55	cd	168	c	96	ab
Second	Leaf	53	a	243	b	246	a	745	a	NA	
Second	Stem	75	a	460	b	264	a	493	b	NA	
Second	Fruit	72	a	181	b	121	b	NA		NA	

[a] ^{14}C-glyphosate at 8,333 Bq plus non-ionic surfactant at 0.25% (v/v) per plant was manually applied in ten 1-uL droplets on the upper most fully expanded leaf of each plant. Applications were made at 0 [day of mepiquat chloride (MC) applications], 7, 14, and 21 d after MC treatment (DMC).

[b] Means within a column followed by the same letter are not different according to LSD test at P=0.05.

[c] All node letters have been arbitrarily defined. Node A is one node below the treated leaf while nodes C, D, and E are one, two, and three nodes above the treated leaf, respectively. Node B is the site of ^{14}C-glyphosate application, with actual nodal values shown in Table 1.

Table 8. Interaction of mepiquat chloride and sympodial position for ^{14}C-glyphosate concentration in the various cotton parts within each position for node A, averaged over trials.[a]

PGR[ab] regime	Position	Part	^{14}C-glyphosate Node A[c]	
			Bq g^{-1} dry tissue	
None	Mainstem	Leaf	136	f
None	Mainstem	Stem	51	bcd
None	First	Leaf	41	f
None	First	Stem	47	f
None	First	Fruit	49	b-e
None	Second	Leaf	50	b-e
None	Second	Stem	60	b-e
None	Second	Fruit	35	c-f
MC	Mainstem	Leaf	38	ef
MC	Mainstem	Stem	57	bc
MC	First	Leaf	47	def
MC	First	Stem	42	ef
MC	First	Fruit	56	bc
MC	Second	Leaf	56	bc
MC	Second	Stem	89	b
MC	Second	Fruit	109	a

[a] Abbreviation: MC, mepiquat chloride; PGR, plant growth regulator.

[b] PGR regimes were no PGR (none) or MC at 49 g ai ha^{-1} applied at first bloom (MC).

[c] Means within a column followed by the same letter are not different according to LSD test at P=0.10.

Table 9. Interaction of ^{14}C-glyphosate timing, mepiquat chloride, and sympodial position for ^{14}C-glyphosate concentration in the various cotton parts within each position, averaged over trials.[a]

Timing[a] DMC	PGR[bc] regime	Position	Part	^{14}C-glyphosate			
				Node C[de]		Node D	
				Bq g^{-1} dry tissue			
0	None	Mainstem	Leaf	71	ef	113	e
7	None	Mainstem	Leaf	38	ef	96	e
14	None	Mainstem	Leaf	61	ef	121	e
21	None	Mainstem	Leaf	74	ef	344	bcde
0	MC	Mainstem	Leaf	42	ef	258	bcde
7	MC	Mainstem	Leaf	64	ef	117	e
14	MC	Mainstem	Leaf	54	ef	128	de
21	MC	Mainstem	Leaf	38	ef	166	bcde
0	None	Mainstem	Stem	58	ef	73	e
7	None	Mainstem	Stem	51	ef	100	e
14	None	Mainstem	Stem	42	ef	89	e
21	None	Mainstem	Stem	50	ef	137	de
0	MC	Mainstem	Stem	56	ef	729	b
7	MC	Mainstem	Stem	58	ef	85	e
14	MC	Mainstem	Stem	54	ef	143	de
21	MC	Mainstem	Stem	131	ef	273	bcde
0	None	First	Leaf	42	ef	68	e
7	None	First	Leaf	59	ef	92	e
14	None	First	Leaf	41	ef	78	e
21	None	First	Leaf	44	ef	85	e
0	MC	First	Leaf	80	ef	225	de
7	MC	First	Leaf	59	ef	86	e
14	MC	First	Leaf	28	f	105	e
21	MC	First	Leaf	43	ef	245	bcde
0	None	First	Stem	68	ef	210	de
7	None	First	Stem	55	ef	159	de
14	None	First	Stem	62	ef	173	de
21	None	First	Stem	81	ef	196	bcde
0	MC	First	Stem	213	cdef	181	bcde
7	MC	First	Stem	114	ef	153	de
14	MC	First	Stem	282	bcde	243	bcde
21	MC	First	Stem	92	cdef	1784	a
0	None	First	Fruit	146	cdef	278	bcde
7	None	First	Fruit	218	cdef	384	bcde
14	None	First	Fruit	206	cdef	643	bcd
21	None	First	Fruit	341	bcd	273	bcde

Table 9 (continued)

0	MC	First	Fruit	81	ef	1603	a
7	MC	First	Fruit	350	bc	222	cde
14	MC	First	Fruit	470	ab	773	bc
21	MC	First	Fruit	162	cdef	1796	a
0	None	Second	Leaf	203	cdef	185	de
7	None	Second	Leaf	348	abcd	466	bcde
14	None	Second	Leaf	164	cdef	423	bcde
21	None	Second	Leaf	220	cdef	210	bcde
0	MC	Second	Leaf	108	def	259	bcde
7	MC	Second	Leaf	364	abc	253	bcde
14	MC	Second	Leaf	567	a	356	bcde
21	MC	Second	Leaf	138	cdef		
0	None	Second	Stem	21	f	44	e
7	None	Second	Stem	50	ef	47	e
14	None	Second	Stem	52	ef	41	e
21	None	Second	Stem	47	ef	56	e
0	MC	Second	Stem	73	ef	47	e
7	MC	Second	Stem	71	ef	52	e
14	MC	Second	Stem	56	ef	61	e
21	MC	Second	Stem	72	ef	63	e
0	None	Second	Fruit	43	ef	55	e
7	None	Second	Fruit	48	ef	48	e
14	None	Second	Fruit	52	ef	46	e
21	None	Second	Fruit	58	ef	70	e
0	MC	Second	Fruit	56	ef	43	e
7	MC	Second	Fruit	66	ef	71	e
14	MC	Second	Fruit	45	ef	67	e
21	MC	Second	Fruit	70	ef	70	e

[a] ^{14}C-glyphosate at 8,333 Bq plus non-ionic surfactant at 0.25% (v/v) per plant was manually applied in ten 1-uL droplets on the upper most fully expanded leaf of each plant. Applications were made at 0 [day of mepiquat chloride (MC) applications], 7, 14, and 21 d after MC treatment (DMC).

[b] Abbreviation: MC, mepiquat chloride; PGR, plant growth regulator.

[c] PGR regimes were no PGR (none) or MC at 49 g ai ha^{-1} applied at first bloom (MC).

[d] All node letters have been arbitrarily defined. Node A is one node below the treated leaf while nodes C, D, and E are one, two, and three nodes above the treated leaf,

Table 9 (continued)

respectively. Node B is the site of ^{14}C-glyphosate application, with actual nodal values shown in Table 1.

[e] Means within a column followed by the same letter are not different according to LSD test at P=0.05.

Translocation of ^{14}C-glyphosate as influenced by various plant growth regulators in cotton.

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Abstract: Studies examined various morphological characteristics and ^{14}C-glyphosate translocation in cotton as influenced by cotton plant growth regulator regimes [none, mepiquat chloride (MC), mepiquat pentaborate (MP)] and source leaf (^{14}C-glyphosate was applied to mainstem and sympodial leaves). Mepiquat chloride and MP reduced cotton height by 13 and 20%, respectively, compared to non-treated plants. Mepiquat chloride and MP reduced the number of mainstem nodes by 1.4 and 1.9 nodes plant^{-1}, respectively, compared to non-treated plants. Total fruit retention, first position fruit retention, low sympodial fruit retention (nodes 6-10), high sympodial fruit retention (nodes 11-15), and the location of the first sympodial branch were not influenced by either ^{14}C-glyphosate treatment or cotton PGR regime. On node 19, MC and MP reduced the leaf area of the mainstem leaf by 59 and 73%, compared to the mainstem leaf of non-treated plants. This observed reduction may be partially influenced by the reduction in cotton height and the number of nodes. Leaf area at other positions was only influenced by node and position within nodes. Absorption of ^{14}C-glyphosate was not influenced by either ^{14}C-glyphosate placement or cotton PGR regime. Based on these data, MC and MP do not influence ^{14}C-glyphosate translocation, which presumably indicates no direct influence on photoassimilate translocation.

Nomenclature: Glyphosate, mepiquat chloride, mepiquat pentaborate, cotton, *Gossypium hirsutum* L.

Key words: Translocation, photoassimilates.

Introduction

Development and retention of cotton fruits are influenced by supply and demand of photoassimilates. The complex balance of these source-to-sink relationships varies by plant position and age (Ashley 1972; Benedict and Kohel 1975) as well as environmental conditions (Guinn 1982). Multiple researchers have investigated photoassimilate patterns in cotton with $^{14}CO_2$ (Ashley 1972; Benedict and Kohel 1975; Horrocks et al. 1978) and ^{14}C-glyphosate (Feng and Chiu 2005). Ashley (1972) and Horrocks et al. (1978) showed that the subtending leaf of a fruit was its primary photoassimilate source. Furthermore, Ashley (1972) showed that at least 88% of the ^{14}C-photoassimilate remained in the treated sympodial branch. With different leaf morphology of normal and superokra leaf cotton, Horrocks et al. (1978) found that the smaller superokra leaves supplied 88% of the photoassimilate to the subtending fruits compared to normal leaves, indicating that photoassimilate translocation patterns are similar regardless of leaf morphological differences.

Glyphosate, a commonly used systemic herbicide, is translocated in the phloem following a source-to-sink relationship (Dewey and Appleby 1983; Sandberg et al. 1980; Tardif and Leroux 1993). Due to these properties, similar patterns of glyphosate and sucrose translocation have been reported in Canada thistle [*Cirsium arvense* (L.) Scop.], cotton, quackgrass [*Elytrigia repens* (L.) Nevski], and tall morningglory [*Ipomoea purpurea* (L.) Roth.] (Dewey and Appleby 1983; Harker and Dekker 1988; Klevorn and Wyse 1984; McAllister and Haderlie 1985; Shieh et al. 1993; Tardif and Leroux 1993; Thomas et al. 2006). After accounting for differences in export between glyphosate and sucrose, Thomas et al. (2006) showed that glyphosate and sucrose were distributed equally,

regardless of cotton growth stage (5- and 10-leaf). Furthermore, Tadrif and Leroux (1993) found that glyphosate and sucrose translocation was not significantly different in three biotypes of quackgrass. Correlations for ^{14}C- glyphosate and ^{3}H-sucrose localization in tips, buds, and rhizome segments of quackgrass were 0.89, 0.93, and 0.98, respectively (Shieh et al. 1993). However, Harker and Dekker (1988) have shown that glyphosate translocation is less than sucrose translocation in quackgrass, but follows a similar translocation pattern. Other research has found similar translocation relationships between photoassimilates and glyphosate, depending on plant species, phenological stage, and environmental stress levels (Dewey and Appleby 1983; Klevorn and Wyse 1984; McAllister and Haderlie 1985). However, glyphosate treatment in susceptible species may limit its own translocation (Geiger and Bestman 1990; Hetherington et al. 1999). In order to utilize glyphosate as a photoassimilate model, genetically engineered glyphosate-resistant plants may be used to limit this phenomenon (Feng and Chiu 2005; Hetherington et al. 1999). A new generation of glyphosate-resistant cotton (GR2) allowed glyphosate application up 7 day before harvest due to reported increased reproductive tolerance. By combining the herbicidal characteristics of glyphosate and glyphosate-resistant technology in cotton, Feng and Chiu (2005) examined glyphosate translocation as influenced by plant position to show similar patterns of translocation from sympodia leaves to adjacent fruits.

Many cotton production systems include the use of plant growth regulators (PGRs) to manage vegetative cotton growth. Mepiquat chloride (MC) and mepiquat pentaborate (MP), two onium-type growth regulators commonly used in these systems, inhibit gibberillic acid synthesis by stopping the conversion of geranylgeranyl diphosphate to *ent*-kaurene, consequently reducing cell enlargement and the rate of cell division (Rademacher

2000; Srivastava 1993). The visual effects of these PGRs included reduced stem and leaf expansion and generally enhanced earliness of cotton (Reddy et al. 1990, 1996; York 1983a, 1983b). However, cotton yield responses were variable (Kerby 1985). Since PGR applications have been shown to alter cotton canopy architecture and fruit maturity, photoassimilate translocation patterns are presumably altered by these applications. Based on increased dry matter partitioning into fruits, Zhao and Oosterhuis (2000) postulated that these observed differences may be due to altered photoassimilate translocation. However, Zhao and Oosterhuis (2000) did not observe any translocation differences of $^{14}CO_2$ from the leaf into the adjacent fruit. Even though research has not confirmed differences in photoassimilate translocation in response to cotton PGR regimes using $^{14}CO_2$ and ^{14}C-sucrose technology, glyphosate and glyphosate-resistant technology offer new tools for investigating photoassimilate translocation patterns in cotton (Feng and Chiu 2005).

Since glyphosate movement has been correlated with sucrose movement in cotton (Thomas et al. 2006) and multiple weed species (Dewey and Appleby 1983; Harker and Dekker 1988; Klevorn and Wyse 1984; McAllister and Haderlie 1985; Shieh et al. 1993; Tardif and Leroux 1993), our objectives were to evaluate translocation of ^{14}C-glyphosate from various source leaves as influenced by multiple cotton PGR regimes.

Materials and Methods

Plant material. An experimental GR2 cotton variety was planted in 30-cm pots (9 liter volume) containing a commercial potting medium[1] and grown in a plastic greenhouse maintained at 28 $\pm$ 2 C constant temperature where natural sunlight were supplemented 4 h

daily with mercury halide lights, providing a 16-h day length. After the 8-lf stage, all plants received 300 mL of fertilizer solution[2] (1.3 cm^2/L) weekly.

^{14}C-Glyphosate Treatments and Sampling. A two-factor factorial treatment arrangement was used. Cotton PGR regimes were either (1) nothing, (2) MC[3] at 12 g ai ha^{-1} at match head square followed by (fb) MC at 37 g ha^{-1} at first bloom, or (3) MP[4] at 29 g ai ha^{-1} at match head square fb MP at 86 g ha^{-1} at first bloom. The second factor, placement of ^{14}C-glyphosate[5], was applied to either (1) the mainstem leaf on the first reproductive branch, (2) the leaf subtending the first fruit on the first reproductive branch, (3) the leaf subtending the second fruit on the first reproductive branch, (4) the mainstem leaf on the fifth reproductive branch, (5) the leaf subtending the first fruit on the fifth reproductive branch, and (6) the leaf subtending the second fruit on the fifth reproductive branch. ^{14}C-glyphosate at 8,333 Bq plus non-ionic surfactant[6] at 0.25% (v/v) per plant was manually applied in 10 –1-uL droplets on the selected leaf of each plant (Pline et al. 2001). All applications were made 21 days after the first bloom on the most mature reproductive branch.

Prior to destructive harvests, all plants were mapped for fruit retention (Jenkins et al. 1990). Total plant height was also recorded. All plants were harvested at 14 d after each ^{14}C-glyphosate treatment and were partitioned into nodes, positions within each sympodial branch, and parts within each position. Each mainstem nodal section contained a leaf and stem section while each reproductive position contained a leaf, stem, and fruit (square/boll). Mature fruits were divided into peduncles/bracts, lint, locules, and seed. Fresh weight, dry weight, and leaf area (where applicable) were recorded for each part. Absorption was determined by rinsing the treated portion of the leaf with 10 ml of a

methanol:water (1:1 v/v) plus a non-ionic surfactant[6] (0.25% v/v) (Askew and Wilcut 2001; Pline et al. 2001). The leaf rinsate was diluted in 10 ml scintillation fluid[7] and radioactivity was quantified with liquid scintillation spectrometry (LSS)[8]. Divided plant parts were dried for 48 h at 70 C, weighed, and combusted with a biological sample oxidizer[9]. Radioactivity in the oxidized samples was quantified by LSS. Due to the large number of samples, only two nodes of each plant were processed for ^{14}C quantification. The selected regions included the treated leaf node and one node above the treated leaf.

Experimental Design and Data Analysis. Studies were arranged in complete randomized blocks with four replications. Studies were repeated in time. Analysis of variance conducted using a mixed model in SAS[10] revealed no trial by treatment interaction, thus data were averaged over trials. Data were analyzed for main effects and interactions. Mean separations were performed on data using LS means at P=0.05.

Results and Discussion

Cotton morphology. Cotton height was not influenced by trial or ^{14}C-glyphosate placement, thus data were averaged over trial and ^{14}C-glyphosate placement. Mepiquat chloride and MP reduced cotton height by 13 and 20%, respectively, compared to non-treated plants (Table 1). Previous research has shown height reduction with MC in variable environmental conditions (Pettigrew and Johnson 2005; Nichols et al. 2003; Reddy et al. 1996; Zhao and Oosterhuis 2000). Averaged over multiple tests conducted between 1979 and 1983, Kerby (1985) reported a 17% reduction in height with 49 g ha^{-1} of MC. In Arkansas, height reduction ranged from 15 to 28% with multiple treatments of MC, depending on year and location (Zhao and Oosterhuis 2000). Sequential treatments of

MC at 24 g ha^{-1} reduced cotton by 26% at 5 wks after pinhead square, compared to non-treated plants (Nichols et al. 2003).

The number of mainstem nodes was not influenced by trial or ^{14}C-glyphosate placement, thus data were averaged over trial and ^{14}C-glyphosate placement. Mepiquat chloride and MP reduced the number of mainstem nodes by 1.4 and 1.9 nodes $plant^{-1}$, respectively, compared to non-treated plants (Table 1). When MC was applied sequentially at 24 g ha^{-1}, the number of mainstem nodes was reduced by 1.5 nodes $plant^{-1}$ (Nichols et al. 2003). Additional reductions in the number of nodes were observed with four treatments of MC at 37 g ha^{-1} (Nichols et al. 2003). However, Zhao and Oosterhuis (2000) did not observe a significant reduction in the number of nodes in response to MC treatments.

Total fruit retention, first position fruit retention, low sympodial fruit retention (nodes 6 to 10), high sympodial fruit retention (nodes 11 to 15), and the location of the first sympodial branch were not influenced by either ^{14}C-glyphosate treatment or cotton PGR regime (data not shown). Even though our data did not show significant differences in fruit retention or placement on the plant, previous research has reported greater retention on more basal fruiting branches and lower retention on more apical fruiting branches following MC treatment (Hake et al. 1991; Kerby et al. 1986). Furthermore, Kerby et al. (1986) reported more harvestable fruits on the first, second, and monopodial positions on nodes 8 to 12 of MC treated plants. With the use of MC at 49 g ha^{-1}, York (1983a) reported greater fruit production (fruit m^{-2}) at 3 of 6 locations. At population of 235,000 plant ha^{-1}, York (1983b) reported a 14% increase in fruit production with the use of MC at 49 g ha^{-1}. In addition, Cook and Kennedy (2000) showed that MC treatment could also

increase fruit retention on the second position of lower sympodial branches in response to varying levels of fruit loss. Based on the increased fruit retention in lower portions of the plant, Cook and Kennedy (2000) postulated that this higher retention would maintain sink strength in the lower portion of the plant canopy, which may consequently limit further fruit develop on higher nodes (Kerby et al. 1986).

Leaf area was not influenced by trial or ^{14}C-glyphosate placement, thus data were averaged over trial and ^{14}C-glyphosate placement (Table 2). On node 19, MC and MP reduced the leaf area of the mainstem leaf by 59 and 73%, compared to the mainstem leaf of non-treated plants. This observed reduction may be partially influenced by the reduction in cotton height and the number of nodes (Table 1). Mepiquat pentaborate treatment also reduced the mainstem leaf area on node 11 compared to non-treated plants. Mepiquat chloride treatments have been reported to reduce leaf area (Reddy et al. 1990, 1996; Zhao and Oosterhuis 2000). Total leaf area of MC treated plants was reduced by 16% compared to non-treated plants (Reddy et al. 1996). In different temperature regimes, Reddy et al. (1990) observed that leaf area was reduced the least in the lowest and highest temperature regimes. In our data, leaf area on all other nodes was not influenced by MC or MP treatment (data not shown). Generally, leaf area decreased with increasing nodal position. Comparing the leaf subtending the first position of each node, a similar decline in leaf size for fruiting positions was observed. In addition to a smaller leaf size for more vertical nodal positions, leaf size on sympodial branches was also smaller with increasing distance from the mainstem, regardless of node. Sadras (1995) has discussed the implications for reduced leaf area with increasing vertical and horizontal position within the plant canopy on fruit distribution. These data did not show any difference in specific

leaf weight in response to cotton PGR regime (data not shown). Other research has shown variable responses of specific leaf weight to MC treatments (Reddy et al. 1990; Zhao and Oosterhuis 2000).

Dry weight of plant parts for each position was not influenced by trial or ^{14}C-glyphosate placement, thus data were averaged over trial and ^{14}C-glyphosate placement for nodes 6, 7, 8, 9, 11, 13, and 14 (Table 2). All other nodes were only influenced by plant position (data not shown). Even though significant differences in leaf area were observed, no differences in leaf dry weight were observed, regardless of node or position. Numerical reductions in mainstem stem dry weight were observed for nodes 6, 7, 8, 9, 11, 13, and 14 of MC and MP treated plants, which is supported by the reductions in cotton height with these cotton PGRs. Mepiquat chloride treated plants produced 39, 31 and 30 % less lint on first position fruits on nodes 6, 7, and 8, respectively. Mepiquat pentaborate treated plants produced 29, 32 and 31 % less lint on first position fruits on nodes 6, 7, and 8, respectively. Similar numerical dry weight reductions in the lint fraction from second position fruits on nodes 6, 7, and 8 were observed. However, significant dry weight increases in the lint fraction were observed on first position fruit on node 11 of MP treated plants and on second position fruit on node 9 of MC treated plants. Numerical increases in seed dry weight were observed on first position fruit on nodes 7, 8, and 9 and second position fruit on node 7 of MC and MP treated plants. York (1983 a, b) showed increases in fruit weight (g fruit^{-1}) ranging from 3 to 10% in response to MC treatment. A more detailed analysis of fruit composition revealed an increase in seed weight with MC treatments, which accounted for 75% of the increase in fruit weight (York 1983a). Zhao and Oosterhuis (2000) reported similar increases in fruit weight and reduced lint fraction

for various MC formulations, depending on year and location. For vegetative parts, Zhao and Oosterhuis showed no differences for dry weight of leaves and petioles (% of total dry weight) between MC formulations and non-treated plants at Carbondale, AR in 1997.

^{14}C-Glyphosate Translocation. Absorption of ^{14}C-glyphosate was not influenced by either ^{14}C-glyphosate placement or cotton PGR regime (data not shown). However, when more mature leaves were treated with ^{14}C-glyphosate, absorption was numerically lower. Feng and Chiu (2005) showed that glyphosate absorption was lower with increasing leaf age. Absorption of ^{14}C-glyphosate was greater than 94% of the applied. After evaluating the ^{14}C-glyphosate content in the treated leaf, all leaves contained at least 65% of the applied, which may be indicative of ^{14}C-glyphosate remaining on the leaf surface due to an inefficient wash technique or fixation in the cuticle. Pline et al. (2001) showed that 10 to 44% of the applied ^{14}C-glyphosate remained in the leaf wash, depending on the treatment timing and harvest interval.

Translocation of ^{14}C-glypohsate was not influenced by trial or cotton PGR regime, thus data were averaged over trials and cotton PGR regime (Tables 4 and 5). Based on these data, MC and MP do not influence ^{14}C-glyphosate translocation, which presumably indicates no direct influence on photoassimilate translocation. Even though no significant differences were observed, numerical differences were observed that parallel previous research (Ashley 1972; Benedict et al 1975; Benedict and Kohel 1975; Feng and Chiu 2005; Horrocks et al. 1978). When ^{14}C-glyphosate was applied to the mainstem leaf and leaf subtending the first position on the most mature reproductive node, six and four percent of the applied ^{14}C-glyphosate was translocated to the first position fruit. The lint fraction of these fruits contained 40 and 30% of the ^{14}C-glyphosate found in the fruit.

When $^{14}CO2$ was applied to a subtending leaf at various times after anthesis, translocation of assimilates was maximized at 13, 25, and 25 d after anthesis for the boll wall, ovule, and lint, respectively (Benedict and Kohel 1975). In addition, the ovule contained the greatest activity at all harvest timings (7, 13,19, 25, 31, and 37 d after anthesis) (Benedict and Kohel 1975). Based on the age of the first position fruit on the mature sympodial branch (35 d after anthesis at harvest), lower accumulation was observed compared to fruits on a more immature node. When ^{14}C-glyphosate was applied to younger mainstem and first position leaves, the first position fruit accumulated 11 and 9% of the applied ^{14}C-glyphosate. Since these fruits were younger (approximately 23 d after anthesis), a greater accumulation was observed in lint and seed fractions. In addition, Benedict et al. (1973) reported a maximum ^{14}C-photosynthate incorporation rate into lint near 30 d after anthesis. Other researchers have described similar photoassimilate demands based on fruit age (Schubert et al. 1986).

Using a similar ^{14}C-glyphosate placement regime, Feng and Chui (2005) have proposed a directional model for photoassimilate movement where glyphosate is translocated to more distal sympodial parts. Feng and Chui (2005) showed that subtending leaf on the first position primarily supplies assimilates to the fruit subtending the second position. Excess glyphosate was exported from the sympodial branch into the downward stream. Furthermore, distinct differences in translocation comparing the mature and immature branches were observed (Feng and Chiu 2005). Based on numerical differences, our data also support the conclusion that more immature sympodial branches export greater amounts of assimilate to plant tissue, due to the low sink strength of these immature tissues. Generally, subtending leaves to fruits are the primary source of

photoassimilates (Ashley 1972; Benedict et al 1975; Benedict and Kohel 1975; Horrocks et al. 1978), but accumulation rates vary by sympodial age, position of the source leaf, and position and sink strength of the fruit (Feng and Chiu 2005).

These data have shown that MC and MP reduce cotton height, the number of mainstem nodes, and leaf expansion, which all consequently increase the compactness of the cotton canopy. These canopy characteristics facilitate insect management and decrease boll rot (Schott and Walter 1991; Edmisten 2004). Even though MC and MP altered canopy architecture, no ^{14}C-glyphosate translocation differences were observed in response to these cotton PGRs. Therefore, MC and MP treatment may be influencing other morphological and physiological properties of cotton plants (Schott and Walter 1991) without directly altering photoassimilate movement.

Sources of Materials

[1] MetroMix 200, Sun Gro Horticulture, 15831 N.E. 8th Street, Suite 100, Bellevue, WA 98008.

[2] Peters Professional 20-20-20 General Purpose, The Scotts Company, 14111 Scottslawn Road, Marysville, OH 43041.

[3] Mepichlor, Micro Flo Company, P. O. Box 772099, Memphis, TN 38117.

[4] Pentia, BASF Corporation, 26 Davis Drive, Research Triangle Park, NC 27709.

[5] ^{14}C-glyphosate G-8392, Sigma Chemical Co., P. O. Box 14508, St. Louis, MO 63178.

[6] Induce (mixture of alkyl polyoxylkane ether, free fatty acids, and isopropanol), Helena Chemical Co., 5100 Popular Avenue, Memphis, TN 38137.

[7] Scintiverse® SX18-4 Universal Liquid Scintillation Cocktail, Fisher Scientific, 1 Regeant Road, Fair Lawn, NJ 07410.

[8] Packard Tri-Carb 2100TR Liquid Scintillation Spectrometer, Packard Instrument Co., 220 Warrenville Rd., Downers Grove, IL 60515.

[9] Model OX500 Biological Material Oxidizer, R. J. Harvey Instrument Corp., 123 Patterson St., Hillsdale, NJ 07642.

[10] [SAS] Statistical Analysis Systems software, Ver. 8., SAS Institute, Inc., Box 8000, SAS Circle, Cary, NC 27513.

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Table 1. Cotton height as influenced by cotton plant growth regulator regimes, averaged over trials and ^{14}C-glyphosate placement.

PGR regime[a]	Height	Nodes
	cm	#
None	115 a	19.4 a
Mepiquat chloride	100 ab	18.0 b
Mepiquat pentaborate	92 b	17.5 b

[a] Cotton plant growth regulator regimes were: (1) none, (2) mepiquat chloride (MC) at 12 g ai ha^{-1} at match head square followed by (fb) MC at 37 g ha^{-1} at first bloom, or (3) mepiquat pentaborate (MP) at 29 g ai ha^{-1} at match head square fb MP at 86 g ha^{-1} at first bloom.

Table 2. Leaf area as influenced by plant position and cotton plant growth regulator regimes, averaged over trials and ^{14}C-glyphosate placement.

Plant position	PGR regime[a]	Leaf area[b]					
		Node[c]					
		11		13		19	
		cm^2					
Mainstem	None	158	a	142	a	41	a
1st position	None	78	c	57	b	2	c
2nd position	None	39	d	24	cd	4	bc
Mainstem	MP	138	b	133	a	11	bc
1st position	MP	74	c	42	bc	4	bc
2nd position	MP	35	de	8	de	.	
Mainstem	MC	166	a	151	a	17	d
1st position	MC	86	c	51	bc	5	bc
2nd position	MC	34	de	8	de	.	

[a] Cotton plant growth regulator regimes were: (1) none, (2) mepiquat chloride (MC) at 12 g ai ha^{-1} at match head square followed by (fb) MC at 37 g ha^{-1} at first bloom, or (3) mepiquat pentaborate (MP) at 29 g ai ha^{-1} at match head square fb MP at 86 g ha^{-1} at first bloom.

[b] Means within a column followed by the same letter are not different according to LSD test at P=0.05.

[c] All other nodes containing sympodial branches (>6) were only significant for plant position effects.

Table 3. Dry weight of plant parts as influenced by plant position and cotton plant growth regulator regimes, averaged over trials and ^{14}C-glyphosate placement.

PGR regime[a]	Plant position	Plant part	Dry weight													
			Node[bcd]													
			6		7		8		9		11		13		14	
			g													
None	Mainstem	Leaf	0.82	i	0.86	g	0.92	hij	0.97	kl	1.09	efg	1.02	bcd	0.93	ab
MP	Mainstem	Leaf	0.87	ij	0.87	g	0.97	ghi	0.87	klm	1.06	efg	1.04	abc	0.88	b
MC	Mainstem	Leaf	0.87	i	0.90	g	1.03	fgh	1.06	jk	1.17	d-g	1.15	ab	0.99	ab
None	Mainstem	Stem	1.22	efg	1.05	fg	1.08	fgh	0.91	kl	0.65	fgh	0.44	f	0.31	f
MP	Mainstem	Stem	1.02	ghi	0.88	g	0.85	h-k	0.70	k-o	0.45	h-m	0.19	k-o	0.12	h-l
MC	Mainstem	Stem	1.08	f-j	0.96	g	1.01	fgh	0.85	klm	0.50	hij	0.26	h-l	0.16	g-i
None	First	Leaf	0.14	l-o	0.34	h-m	0.39	k-n	0.47	m-q	0.49	h-l	0.39	f-i	0.28	f
MP	First	Leaf	0.28	klm	0.30	i-m	0.42	j-n	0.44	m-s	0.53	hik	0.27	g-k	0.17	g-j
MC	First	Leaf	0.09	m-p	0.33	h-m	0.47	i-n	0.65	l-p	0.56	hik	0.36	f-j	0.24	fgh
None	First	Stem	0.20	lmn	0.31	i-m	0.27	mn	0.34	o-r	0.29	j-s	0.21	j-m	0.14	h-k
MP	First	Stem	0.09	l-p	0.36	h-l	0.24	lmn	0.27	p-r	0.26	j-r	0.10	m-r	0.06	klm
MC	First	Stem	0.01	m-p	0.21	j-m	0.30	lmn	0.37	n-r	0.26	l-s	0.17	k-r	0.07	j-m
None	First	Lint	3.40	a	3.88	a	3.49	a	3.12	a	1.73	b	0.75	e	0.56	e
MP	First	Lint	2.40	b	2.64	b	2.40	b	2.80	abc	2.37	a	0.77	de	0.30	fgh

Table 3 (continued)

MC	First	Lint	2.09	b	2.69	b	2.46	b	2.60	bcd	1.46	bc	0.81	e	0.68	de
None	First	Seed	2.12	bc	1.39	c-g	2.23	bcd	2.20	cde	1.26	cde	0.97	a-e	NE	
MP	First	Seed	2.12	b	2.18	bc	2.32	bc	1.98	d-g	1.18	b-f	NE		NE	
MC	First	Seed	1.89	bcd	1.59	c-f	2.38	bc	2.23	cde	1.18	c-g	1.35	ab	NE	
None	First	Bracts	0.12	l-o	0.37	h-l	0.31	lmn	0.34	o-r	0.26	j-s	0.42	fgh	0.24	fgh
MP	First	Bracts	NE[d]		0.19	j-m	1.21	fgh	0.27	pqr	0.34	i-o	0.23	f-q	0.21	f-k
MC	First	Bracts	0.07	m-p	0.24	j-m	0.28	lmn	0.38	n-r	0.27	k-s	0.18	k-r	0.24	fgh
None	First	Locules	1.40	de	1.86	cd	1.78	cde	1.68	e-h	1.40	cd	1.19	a	0.82	bcd
MP	First	Locules	1.21	e-h	1.48	de	1.49	def	1.43	f-j	1.51	bc	0.83	cde	0.90	abc
MC	First	Locules	1.09	e-i	1.34	ef	1.40	efg	1.56	f-i	1.20	de	1.15	ab	0.73	cde
None	Second	Leaf	NE		0.30	i-m	0.29	lmn	0.33	o-r	0.23	m-q	0.15	k-r	0.08	i-m
MP	Second	Leaf	NE		0.12	klm	0.26	lmn	0.29	o-r	0.21	n-q	0.04	p-s	0.02	i-m
MC	Second	Leaf	NE		0.26	j-m	0.38	k-n	0.40	n-r	0.20	m-q	0.05	n-s	0.00	lm
None	Second	Stem	NE		0.16	klm	0.11	n	0.14	r	0.09	o-t	0.04	o-s	0.02	m
MP	Second	Stem	NE		NE		0.07	n	0.07	uv	0.04	q-t	0.01	rs	0.00	klm
MC	Second	Stem	NE		0.09	klm	0.09	n	0.15	qr	0.05	p-t	0.01	qrs	NE	
None	Second	Lint	1.49	c-f	1.41	d-g	1.44	d-h	1.01	i-l	0.58	f-q	0.22	f-r	0.05	f-m
MP	Second	Lint	1.06	e-i	1.22	efg	1.27	e-h	1.29	f-l	NE		NE		NE	
MC	Second	Lint	0.96	e-i	0.93	e-j	0.89	f-m	3.14	abc	0.58	d-q	NE		NE	

Table 3 (continued)

None	Second	Seed	1.17	d-i	0.55	e-m	1.24	c-m	1.25	e-q	NE		NE		NE	
MP	Second	Seed	0.80	g-k	1.13	efg	1.01	e-m	0.62	h-r	NE		NE		NE	
MC	Second	Seed	0.72	g-l	1.30	c-k	0.95	e-n	1.21	e-r	NE		NE		NE	
None	Second	Bracts	NE		0.14	j-m	0.07	mn	0.15	o-r	0.24	h-q	0.10	l-r	0.02	g-m
MP	Second	Bracts	NE		0.05	lm	0.08	mn	0.19	n-t	NE		NE		NE	
MC	Second	Bracts	NE		0.11	h-m	0.09	lmn	0.72	j-r	0.07	h-q	0.04	f-r	0.01	f-m
None	Second	Locules	0.90	ghi	0.97	e-h	1.07	f-j	0.91	j-n	1.09	c-f	0.20	f-r	0.01	f-m
MP	Second	Locules	0.82	hi	1.02	fg	1.05	f-k	1.11	g-m	NE		NE		NE	
MC	Second	Locules	0.99	e-i	1.05	e-i	0.96	f-l	2.19	c-f	0.69	c-q	0.23	f-r	0.02	f-m

[a] Cotton plant growth regulator regimes were: (1) none, (2) mepiquat chloride (MC) at 12 g ai ha^{-1} at match head square followed by (fb) MC at 37 g ha^{-1} at first bloom, or (3) mepiquat pentaborate (MP) at 29 g ai ha^{-1} at match head square fb90 MP at 86 g ha^{-1} at first bloom.

[b] Means within a column followed by the same letter are not different according to LSD test at P=0.05.

[c] All other nodes containing sympodial branches (>6) were only significant for plant position effects.

[d] The estimate for plant parts was not calculable using a mixed model.

Table 4. Translocation of ^{14}C-glyphosate as influenced by plant position, averaged over trials, ^{14}C-glyphosate placement, and cotton plant growth regulator regimes. Data are from the node containing the treated leaf.

Position	Plant part	^{14}C-glyphosate treatment location[a]					
		Mature sympodial branch			Immature sympodial branch		
		Treated leaf					
		Mainstem	1st position	2nd position	Mainstem	1st position	2nd position
		% of applied					
Mainstem	Leaf	78.22 a	0.23 b	0.32 b	65.46 a	2.94 b	5.15 b
Mainstem	Stem	1.44 b	0.47 b	0.32 b	0.28 b	0.21 b	0.25 b
First	Bract	0.60 b	0.59 b	0.21 b	0.40 b	0.73 b	0.09 b
First	Leaf	NE[b]	70.47 a	0.17 b	3.85 b	71.78 a	0.25 b
First	Locules	1.28 b	1.57 b	0.59 b	1.18 b	2.18 b	0.99 b
First	Lint	2.36 b	1.33 b	0.58 b	2.42 b	2.58 b	0.59 b
First	Stem	0.20 b	0.62 b	0.13 b	NE	0.52 b	NE
First	Seed	1.67 b	1.54 b	0.50 b	2.42 b	2.83 b	0.94 b
Second	Bract	NE	0.35 b	1.40 b	NE	NE	NE
Second	Leaf	0.63 b	0.29 b	71.47 a	1.11 b	8.10 b	76.29 a
Second	Locules	NE	1.02 b	1.45 b	NE	NE	NE
Second	Lint	0.19 b	1.02 b	1.70 b	NE	NE	NE
Second	Stem	0.04 b	0.05 b	0.79 b	NE b	0.12 b	0.55 b
Second	Seed	0.65 b	1.24 b	1.89 b	NE .	NE	NE

[a] Means within a column followed by the same letter are not different according to LSD test at P=0.05.

[b] The estimate for plant parts was not calculable using a mixed model.

Table 5. Translocation of ^{14}C-glyphosate as influenced by plant position, averaged over trials, ^{14}C-glyphosate placement, and cotton plant growth regulator regimes. Data are from one node above the treated leaf node.

Position	Plant part	^{14}C-glyphosate treatment location[a]					
		Mature sympodial branch			Immature sympodial branch		
		Treated leaf					
		Mainstem	1st position	2nd position	Mainstem	1st position	2nd position
		% of applied					
Mainstem	Leaf	0.51 ab	0.67 a	0.84 b	6.86 ab	0.65 ab	3.09 a
Mainstem	Stem	0.43 b	0.39 a	0.66 b	0.15 b	0.22 cd	0.62 b
First	Bract	0.11 b	0.18 a	0.69 ab	0.18 ab	NE	0.21 b
First	Leaf	1.55 ab	4.69 a	0.38 b	8.63 a	0.22 cd	0.43 b
First	Locules	0.51 ab	0.57 a	1.78 b	0.80 ab	NE	0.61 b
First	Lint	0.88 a	2.69 a	1.56 b	0.61 ab	NE	0.69 b
First	Stem	0.13 b	0.19 a	0.19 b	0.12 b	0.10 cde	0.16 b
First	Seed	0.75 ab	1.20 a	1.25 ab	1.14 ab	NE	0.61 b
Second	Bract	NE[b]	0.16 a	1.52 ab	NE	NE	NE
Second	Leaf	0.12 b	0.47 a	23.82 a	0.19 ab	0.10 cde	0.11 b
Second	Locules	NE	0.32 a	3.38 ab	NE	NE	NE
Second	Lint	NE	0.26 a	6.60 ab	NE .	NE	NE
Second	Stem	0.03 b	0.03 a	0.46 b	0.02 ab	0.02 ef	0.03 b
Second	Seed	NE	0.33 a	NE	NE	NE	NE

[a] Means within a column followed by the same letter are not different according to LSD test at P=0.05.

[b] The estimate for plant parts was not calculable using a mixed model.

Rain-Free Requirement and Physiological Properties of Cotton Plant Growth Regulators

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Abstract

Greenhouse studies were conducted to (1) evaluate the rain-free requirement for mepiquat chloride and mepiquat chloride plus cyclanilide with and without surfactant and to (2) evaluate absorption and translocation of cyclanilide, a component of a new cotton plant growth regulator. No significant differences in the number of nodes, leaf area, and plant part fresh and dry weight were observed with any PGR treatment and rainfall simulation combination. Both plant growth regulators responded similarly to rainfall interval. As rain-free period increased, cotton height was reduced. Based on these data, a rain-free period of 8 hours is needed to maximize efficacy, regardless of the use of surfactant. Absorption of cyclanilide ranged from 11 to 15% at 3 and 48 HAT, respectively. Averaged over harvest intervals, 18% of the applied cyclanilide remained in the treated leaf while 1.7 and 6.5% of the applied cyclanilide was found in the above and below treated leaf tissue, respectively.

Keywords: plant height, absorption, translocation, rain-free period, leaf area.

1. Introduction

Many cotton (*Gossypium hirsutum*) production systems include the use of plant growth regulators[6] to manage vegetative growth. Mepiquat chloride, an onium-type growth regulator commonly used in cotton, inhibits gibberillic acid synthesis by stopping the conversion of geranlgeranyl diphosphate to *ent*-kaurene, consequently reducing cell enlargement and the rate of cell division [1, 2]. The visual effects of these PGRs include reduced stem and leaf expansion and generally enhanced earliness of cotton [3-6].

[6] PGRs, plant growth regulators.

Mepiquat chloride treatment can also facilitate insect management and decrease boll rot [4, 7]. However, yield responses to mepiquat chloride are often variable [8].

Multiple mepiquat chloride treatments at various application rates are commonly used to manage cotton growth [4, 7]. Due to the interactions of environmental conditions with mepiquat chloride treatments [7], recommendations for treatment to cotton vary widely by geographic region. A new prepackaged mixture of mepiquat chloride and cyclanilide has been reported to limit the variability in application rate while maintaining plant growth regulation [9, 10]. Cyclanilide is amalonanilate with a cyclopropane ring similar to 1-aminocyclopropane-1-carboxylic acid [11]. Compared to known polar auxin transport inhibitors (1-*N*-naphthylphthalamic acid[7] and 2, 3, 4-triiodobenzoic acid[8]) [12], Pederson [13] reported similar levels of inhibition of auxin transport with 10 ? m of cyclanilide in etiolated coleoptiles of corn (*Zea mays*). Due to limited basipetal transport of auxin in cyclanilide treated plants, apical dominance is not maintained, allowing for increased lateral shoot growth [13-17]. Thus the mixture of mepiquat chloride and cyclanilide inhibits gibberillic acid synthesis and auxin transport, respectively. Due to the dual modes of action, this prepackaged mixture has been shown to regulate cotton growth with limited variability in application rate compared to mepiquat chloride alone treatments [9, 10].

Since the prepackaged mixture of mepiquat chloride plus cyclanilide has recently been registered, limited data are available with regard to rain-free requirements and fate within cotton. Therefore, our objectives were (1) to determine the rain-free interval for mepiquat

[7] NPA, 1-*N*-naphthylphthalamic acid.

[8] TIBA, 2, 3, 4-triiodobenzoic acid.

chloride and mepiquat chloride plus cyclanilide alone or in combination with a non-ionic surfactant and (2) to evaluate absorption and translocation of cyclanilide in cotton.

2. Materials and Methods

2.1 Plant material

Stoneville '4892 RR/BG' cotton was planted in 30-cm pots containing a commercial potting medium (Metro Mix 200, Sun Gro Horticulture) and grown in a plastic greenhouse maintained at 28 $\pm$ 2 C constant temperature where natural sunlight was supplemented 4 h daily with mercury halide lights, providing a 16-h day length. Studies were conducted from July to November 2005.

2.2 Determination of rain-free requirement

A factorial treatment arrangement of cotton PGRs (2 levels) and simulated rainfall timings (8) was used. Cotton was treated at matchhead square, which corresponded with 10- to 11-leaf growth stage, with a prepackaged mixture of mepiquat chloride at 16.1 g ai ha^{-1} plus cyclanilide at 3.9 g ai ha^{-1} postemergence over the top (Stance, Bayer CropScience) or mepiquat chloride at 24.5 g ai ha^{-1} postemergence over the top (MepiChlor, MicroFlo). In a second study, non-ionic surfactant (Induce, Helena Chemical Co.) at 0.25% (v/v) was added to both PGR treatments. An apparatus was designed using HH-SS50WSQ nozzles to deliver a simulated rainfall at 7.6 cm hr^{-1} at 207 kPa. Simulated rainfall was applied for 10 min at 0.25, 0.5, 1, 2, 4, 8, and 24 h after PGR treatment. PGR-treated plants with no rainfall and no PGR-treated plants were included for comparison.

On the day of PGR treatment, all plants were marked at the third and fifth nodes from the apex. Plant height was recorded at 0, 7, and 14 d after PGR treatment from the soil to

the plant apex and from the marked nodal positions to the apex. At 14 d after PGR treatment, all plants were destructively harvested by node and parts within nodes. Within each node, parts included mainstem leaf, mainstem stem, reproductive/vegetative leaves, reproductive/vegetative stems, and reproductive/vegetative fruits. Fresh and dry weights were recorded for all parts while mainstem nodal length and leaf area were recorded where applicable.

2.3 Absorption and translocation of cyclanilide

Cotton was treated at matchhead square, which corresponded with 10- to 11-leaf growth stage with a prepackaged mixture of mepiquat chloride at 16.1 g ai ha^{-1} plus cyclanilide at 3.9 g ai ha^{-1} postemergence over the top. Prior to PGR treatment, a 20 cm^3 area of the newest completely expanded leaf was covered with aluminum foil to prevent spray contact with the leaf surface. The covered area of each plant was treated with 10-one ? l droplets containing 8.3 kBq of ^{14}C-cyclanilide (cyclanilide, specific activity **7.5** ? ? q mg^{-1}, Bayer CropScience).

Plants were harvested at 3, 8, 24, 48, 72, 168, 336, and 504 h after treatment. Absorption was determined by rinsing the treated portion of the leaf with 10 ml of a methanol:water (1:1, v/v) plus 0.25% non-ionic surfactant (Induce, Helena Chemical Co.) [17] and diluted in 15 ml scintillation fluid (Ultima Gold, PerkinElmer) and radioactivity was quantified with liquid scintillation spectrometry (Tri-Carb 2100TR, Packard Instruments). All plants were then divided into four regions: 1) treated leaf, 2) above treated leaf, 3) below treated leaf, and 4) roots. The treated leaf was removed at the point of attachment to the stem, which determined the division for above and below the treated leaf sections. The mainstem tissue above and below the treated leaf were each divided into

leaves and stems. The reproductive branches above and below the treated leaf were each divided into leaves, stems, and fruits. Plant parts were dried for 48 h at 70 C for 2 d, weighed, homogenized, and combusted with a biological sample oxidizer (OX-500, R. J. Harvey Instrument Co.). Radioactivity in the oxidized samples was quantified by liquid scintillation spectrometry.

2.4 Experimental design and data analysis

Studies were arranged in randomized complete blocks. Rain-free requirement and ^{14}C-cyclanilide studies contained four and three replications of treatments, respectively, and were repeated in time. All data were subjected to analysis of variance. Trials were averaged due to insignificant trial interactions. Significant simulated rainfall and absorption effects were observed, thus regression analysis was performed. Nonlinear models were used if ANOVA indicated higher-order polynomial effects were more significant than linear effects. Iterations were performed to determine parameter estimates with least sums of squares for all nonlinear models using the Gauss-Newton method via PROC NLIN in SAS [18].

3. Results and Discussion

3.1 Determination of rain-free interval

Since no differences were observed between trials, all data were averaged over trials. No significant differences in the number of nodes, leaf area, and plant part fresh and dry weight were observed with any PGR treatment and rainfall simulation combination (data not shown). Regardless of the use of surfactant, no significant differences in plant height

were observed with mepiquat chloride alone or in combination with cyclanilide, indicating that these two commercial products have similar rain-free requirements (data not shown). However, significant reductions in plant height, which are a primary indicator of plant growth regulator efficacy in cotton, were observed for the simulated rainfall effect in no surfactant and surfactant studies (Table 1 and Figure 1). The change in total plant height, measured as the difference in growth between 0 to 7 d and 7 to 14 d after application, showed a significant response to rainfall interval, regardless of the inclusion of surfactant. As the rain-free period increased, plant height was reduced. Averaged over PGR treatments without surfactant, the change in total plant height at 0 to 7 d was reduced by 5 and 19% with simulated rainfall at 4 and 8 h after treatment, respectively, compared to no PGR-treated plants. Similarly when surfactant was added, the change in total plant height at 0 to 7 d after treatment was reduced by 1 and 19% with a simulated rainfall at 4 and 8 h after treatment, respectively, compared to no PGR-treated plants.

Since the apical regions of cotton contain the majority of expanding leaf and stem tissue, the growth rates of the 5 and 3 node apical regions were similar to the total growth rate as evidenced by regression parameters (Table 1). Plants treated with either PGR did not have continued growth reduction, which is evidenced by the variability in growth rates at 7 to 14 d after treatment. These data support previous research documenting the need for multiple applications of PGRs to adequately manage vegetative growth of cotton in optimum environmental conditions [4, 7]. Based on these data, a rain-free period of 8 h is required for both PGRs evaluated, regardless of the inclusion of a surfactant.

3.2 ^{14}C-Cyclanilide Absorption and Translocation

Absorption of ^{14}C-cyclanilide by cotton was not influenced by trial, thus data were averaged over trials (Figure 1). Absorption, as a percentage of the applied ^{14}C-cyclanilide, increased over time and ranged from 11 to 15% at 3 and 48 HAT, respectively. Absorption after 48 h was variable (53 to 67%). Other research has shown less than 7% absorption of cyclanilide in cotton [20]. The observed increase in absorption of cyclanilide after 48 HAT may be due to cuticlar binding. Previous research with cyclanilide included a 3 min chloroform wash [20], which probably removed most of the cuticle and potentially the bound cyclanilide. In addition, Collins [20] did not evaluate the long term physiological behavior of cyclanilide.

Translocation of ^{14}C-cyclanilide was not significantly different between trials, thus data were averaged over trials. Even though harvest timings main effects and the harvest timing by plant part interaction were significant, only the plant part factor will be discussed due to the large F value compared to the other parameters (Table 2). Translocation of ^{14}C-cyclanilide is shown as a percentage of the applied radioactivity (Table 3). The treated leaf contained the greatest amounts of cyclanilide (18%). Mainstem leaves below the treated leaf contained more cyclanilide than mainstem stems and reproductive leaves, stems, and fruit above the treated leaf. Numerically, 1.7 and 6.5% of the applied cyclanilide was found above and below the treated leaf, respectively. The observed translocation patterns of cyclanilide are similar to basipetal translocation of auxin [15]. Even though levels of cyclanilide were significantly different based on the applied dose, no differences in cyclanilide translocation out of the treated leaf were observed when the accumulation was based on the level of activity per unit of dry matter (Table 3). With the two data presentations, the low translocation of cyclanilide may be of limited biological

significance. Since cyclanilide inhibits auxin transport and hormones are biologically active at extremely low concentrations, only relatively small quantities of cyclanilide may be needed to provide this inhibitory role. For example, Pederson [12] showed that cyclanilide at 10 ? m could effectively inhibit auxin transport in etiolated coleoptiles of corn. These small quantities may be below the level of detection limits with the radiolabel techniques used. In previous research, no translocation of cyclanilide in 5-leaf cotton was reported [20].

4. Conclusions

Auxin has been implicated in a number of plant processes, including induction of cell division, stem elongation, and apical dominance [15]. One major role of gibberillic acid is promotion of cell elongation [2]. Inhibiting cell elongation through alterations of the physiological properties of multiple plant hormones may provide more consistent plant height reductions compared to inhibiting only gibberellic acid synthesis. In addition, cyclanilide may increase the number of lateral fruiting branches as reported in apples [14]. Field data comparing these two PGRs support these observations [9, 10].

Based on plant height data, the primary indicator of PGR efficacy in cotton, an 8-h rain-free period is required for optimum performance of mepiquat chloride and mepiquat chloride plus cyclanilide, regardless of the inclusion of surfactant. Comparing these two commercial products, no significant differences were noted with regard to cotton height reductions. Therefore, mepiquat chloride and mepiquat chloride plus cyclanilide provide equivalent cotton height reductions with similar rain-free period requirements. However,

additional research is needed to evaluate the efficacy of mepiquat chloride and mepiquat chloride plus cyclanilide in optimum and sub-optimum environmental conditions.

In the ^{14}C-cyclanilide studies, at least 15% of the applied cyclanilide was absorbed at 48 h after treatment. However, limited translocation (< 3% of applied dose) to individual plant parts was observed with the greatest amount being transported to mainstem leaves below the treated leaf (2%). The basipetal direction and low levels of translocation of cyclanilide may partially explain the need for multiple applications of PGRs to adequately limit vegetative growth of cotton in optimum environmental conditions. Additional physiological studies should be conducted to investigate the basis for synergism of mepiquat chloride and cyclanilide in cotton.

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Table 1

Parameters (standard error) for regression analysis [y = -a ln(time) + b] on the change in cotton height in response to various simulated rainfall timings.

Surfactant[ab]	Time[c]	Measurement	a	SE[d]	b	SE	R^{2}[e]
None	0 to 7 d[1]	Total[f]	0.892	0.139	14.29	0.229	0.89
None	0 to 7 d[2]	5 apical nodes	0.875	0.138	14.69	0.229	0.89
None	0 to 7 d[3]	3 apical nodes	0.633	0.118	12.56	0.195	0.85
NIS	0 to 7 d[4]	Total[f]	0.736	0.263	17.11	0.435	0.61
NIS	0 to 7 d[5]	5 apical nodes	0.602	0.250	16.54	0.414	0.56
NIS	0 to 7 d[6]	3 apical nodes	0.589	0.285	14.58	0.471	0.46
None	7 to 14 d[7]	Total[f]	0.267	0.145	11.31	0.240	0.41
None	7 to 14 d[8]	5 apical nodes	0.301	0.156	12.02	0.259	0.43
None	7 to 14 d[9]	3 apical nodes	0.289	0.177	12.05	0.292	0.35
NIS	7 to 14 d[10]	Total[f]	0.142	0.725	13.79	1.20	0.01
NIS	7 to 14 d[11]	5 apical nodes	0.164	0.711	13.69	1.18	0.02
NIS	7 to 14 d[12]	3 apical nodes	0.179	0.697	13.44	1.16	0.02

[a] Abbreviation: NIS, non-ionic surfactant.

[b] A non-ionic surfactant at 0.25% (v/v) was included.

Table 1 (continued)

[c] Means (cm) for non-treated and no-rain controls were: [1]14.8, 12.0; [2]15.7, 12.7; [3]13.5, 9.7; [4]17.5, 14.1; [5]17.3, 14.1; [6]14.8, 11.7; [7]13.5, 9.3; [8]13.2, 9.1; [9]13.2, 9.8; [10]16.2, 12.3; [11]16.2, 12.3; and [12]16.1, 12.1, respectively.

[d] Standard error of the estimated parameter.

[e] Using treatment means, R^2 is a percent of the sum of squares for rainfall intervals.

[f] Regression lines for total plant height are shown in Figure 1.

Table 2

F-values and probabilities of the main effects and interactions evaluated for translocation data.

	Translocation			
	% of applied		Bq g^{-1} dry tissue	
	F Value	Pr > F	F Value	Pr > F
Time	1.23	0.3950	9.51	0.0041
Plant part	616.5	<0.0001	112.5	<0.0001
Time*Plant part	15.16	<0.0001	3.60	<0.0001

Table 3

Translocation of ^{14}C-cyclanilide to various plant parts, averaged over trials, and harvest intervals.

Plant part	Translocation[a]			
	Mean	SEM[b]	Mean	SEM[b]
	—— % of applied[c] ——		— Bq g^{-1} dry tissue —	
Treated leaf	17.53	2.00	666.54	64.05
ATL – mainstem leaves	0.76	0.12	11.83	0.84
ATL – mainstem stems	0.25	0.04	11.86	0.80
ATL – reproductive leaves	0.36	0.07	16.30	1.70
ATL – reproductive stems	0.09	0.02	15.77	1.34
ATL – reproductive fruit	0.19	0.04	13.67	1.09
BTL – mainstem leaves	2.16	0.24	15.02	1.47
BTL – mainstem stems	1.75	0.24	15.87	2.55
BTL – reproductive leaves	1.16	0.16	13.34	1.50
BTL – reproductive stems	0.43	0.07	14.81	3.01
BTL – reproductive fruit	0.14	0.03	12.97	1.53
Roots	0.82	0.16	14.92	2.89
Leaf wash	58.03	3.83		
LSD	1.82		49.7	
Recovery	83.7			

[a] Fisher's Protected LSD test at P=0.05.

[b] Abbreviations: SEM, standard error of the mean.

[c] Percent translocation to various plant parts as a percentage of the applied ^{14}C-cyclanilide.

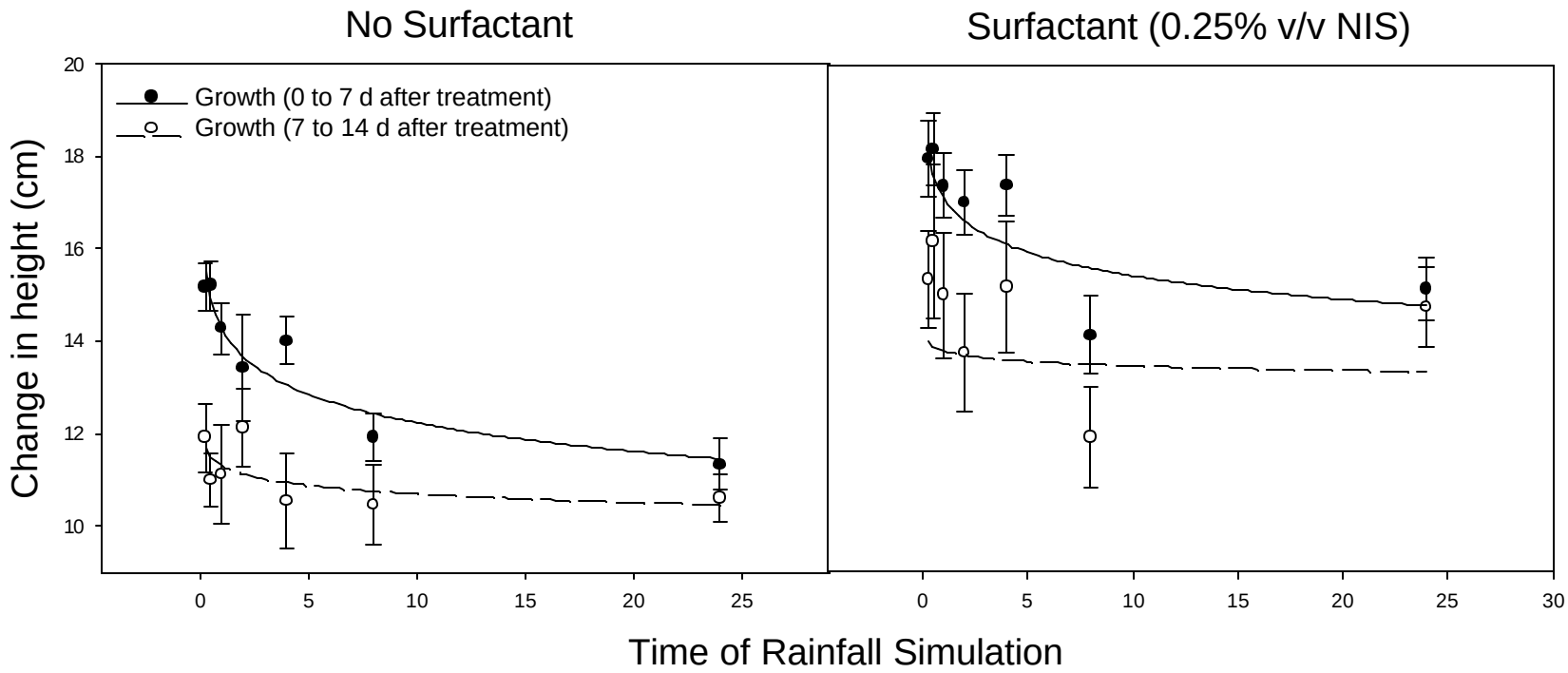

Figure 1. The change in total plant height from 0 to 7 and 7 to 14 d after treatment for no surfactant and surfactant studies, averaged over runs and cotton plant growth regulators. Data modeled using $y = -a \ln(time) + b$. Parameters for equations are shown in table 1.

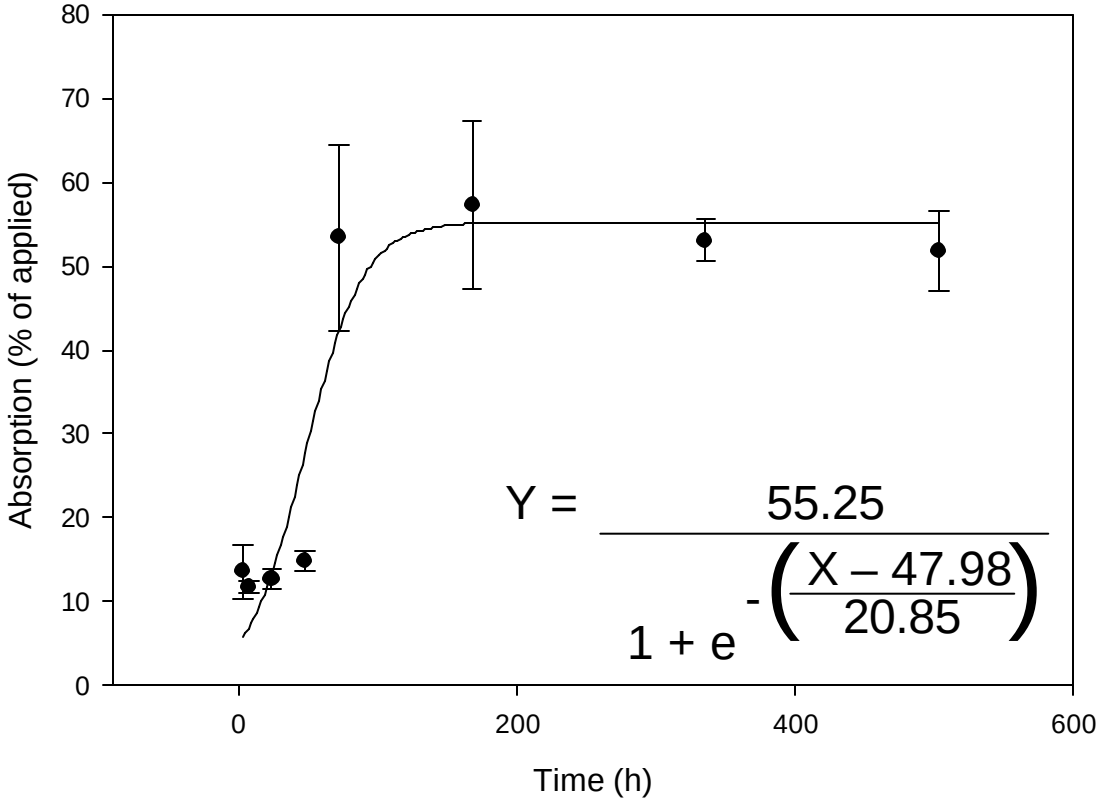

Figure 2. Absorption of ^{14}C-cyclanilide as a percentage of applied, averaged over trials.