

# Yield and Physiological Response of Nontransgenic Cotton to Simulated Glyphosate Drift

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## Abstract

Field studies were conducted in 2001 in Lewiston-Woodville, NC and in 2002 at Clayton and Lewiston-Woodville, NC to investigate the response of nontransgenic cotton to simulated glyphosate drift in a weed-free environment. Nontransgenic cotton variety 'Fibermax 989' was planted in a conventional seedbed at all locations. Glyphosate treatments were applied early postemergence (EPOST) at the 4-leaf growth stage of cotton at 0, 8.7, 17.5, 35, 70, 140, 280, 560, and 1,120 g ai/ha and represents 0, 0.78, 1.55, 3.13, 6.25, 12.5, 25, 50, and 100% of the commercial use rate, respectively. Rates as low as 140 g/ha caused lint yield reductions depending on year and location. When averaged over all locations, lint yield reductions of 4, 49, 72, and 87% compared with nontreated cotton were observed with glyphosate rates of 140, 280, 560, and 1,120 g/ha, respectively. Visual injury and shikimic acid accumulation were evident at glyphosate rates greater or equal to 70 g/ha. Collectively, visual injury and shikimic acid accumulation at 7 DAT might be used as a diagnostic indicator to predict potential yield reductions from simulated glyphosate drift.

## Introduction

Glyphosate-resistant soybean and cotton are common throughout the southeastern portions of United States, providing broad-spectrum control of numerous weed species infesting this warm humid region (Askew and Wilcut 1999; Culpepper et al. 2000). The large hectares of these herbicide-resistant crops increases the potential for glyphosate drift onto neighboring susceptible crops.

Glyphosate mimics the substrate phosphoenolpyruvate, binding to 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme [EC 2.5.1.19] which consequently inhibits the biosynthesis of the aromatic amino acids, tryptophan, tyrosine, and phenylalanine (Siehl 1997). With the inhibition of the EPSPS, shikimate-3 phosphate accumulates and is cleaved to shikimic acid in the tonoplast or vacuole (Hollander-Czytko and Amrhein 1983). Since shikimic acid only accumulates following treatment with glyphosate, it may also exclude other potential herbicidal mode of actions for detrimental crop injury and potential yield losses (Singh and Shaner 1998).

Previous research has investigated simulated drift of glufosinate, glyphosate (Ellis and Griffin 2002), quinclorac (Snipes et al. 1992), and triclopyr (Snipes et al. 1991) in cotton. Since glyphosate causes shikimic acid accumulation after absorption in sensitive plants, we hypothesized that an inverse correlation between yield, and crop injury and shikimic acid accumulation would occur. Therefore, our objectives were 1) to evaluate crop injury, 2) shikimic acid accumulation, and 3) cotton lint yield in nontransgenic cotton treated with eight increasing rates of glyphosate applied at the 4-leaf growth stage.

## Methods

Field studies were established at the Peanut Belt Research Station near Lewiston-Woodville, NC in 2001 and 2002 and at the Central Crops Research Station near Clayton, NC in 2002. Soils were Norfolk sandy loams (fine-loamy, siliceous, thermic, Typic Paleudults) with a pH of 5.8 and 1.1% organic matter at Lewiston-Woodville and a pH of 5.9 and 1.0% organic matter at Clayton.

Nontransgenic cotton variety 'Fibermax 989' was planted into a conventional seedbed at a rate of 13 seed/m of row on May 15, 2001 in Lewiston, April 25, 2002 in Clayton, and May 14, 2002 in Lewiston.

Cotton was seeded in 96 cm and 91 cm row spacing in Clayton and Lewiston, respectively, with a plot length of 6.1 m and 4 rows wide. Pendimethalin at 0.84 kg ai/ha and fluometuron at 1.12 kg ai/ha were applied preemergence (PRE) on the day of planting at each location. An EPOST treatment of glyphosate was applied to cotton at the 4-leaf growth stage to the center two rows of each plot. Glyphosate rates included 0, 8.7, 17.5, 35, 70, 140, 280, 560, and 1,120 g ai/ha and represent 0, 0.78, 1.55, 3.13, 6.25, 12.5, 25, 50, and 100% of the commercial use rate, respectively. A late postemergence-directed (PDS) application of prometryn at 1.12 kg ai/ha plus MSMA at 2.24 kg ai/ha plus a non-ionic surfactant at 0.25% (v/v) followed by a late POST application of clethodim at 140 g ai/ha plus 1.0% (v/v) crop oil concentrate was applied to all plots to control weeds. All plots received biweekly hand weeding to maintain a weed-free environment through harvest.

Visual estimates of cotton injury based on a summation of stunting, discoloration, and stand reduction ranged from 0 (no injury) to 100 (plant death) (Frans et al. 1986). Cotton injury was recorded 7 d after the EPOST treatment (DAT) at all locations. The center two rows of each plot were harvested once with a spindle picker modified for small plot research.

**Shikimic Acid Accumulation.** A modified spectrophotometric method for detection of shikimic acid was used due to the relative simplicity of the laboratory procedures compared to HPLC detection methods (Pline et al. 2002; Singh and Shaner 1998). The spectrophotometric method has been shown to become less efficient at higher shikimic acid concentrations, but plants exhibiting these high accumulation values resulting from high rates of glyphosate are most often killed (Pline et al. 2002). One leaf disc was removed from the third newest leaf of ten plants in the center two rows of each plot using a hole punch. The ten discs were placed in microcentrifuge tubes containing 0.5 mL of 0.01 M H<sub>2</sub>SO<sub>4</sub> and placed on ice while transported back to the laboratory. Once in the laboratory, the samples were ground and 0.25 mL of 0.4 M NaH<sub>2</sub>CO<sub>3</sub> was added to each sample. Solutions remained in -20 °C freezer storage until assay. Samples were allowed to thaw for one hour and then centrifuged at 10,000 g for 5 minutes. After centrifuging, 20 µL of the non-diluted sample was analyzed according to the methods of Singh and Shaner (1998) using a spectrometer at 380 nm. A standard curve was developed using pure shikimic acid standards with known concentrations (Pline et al. 2002). The µg shikimic acid/g plant tissue from all plants was determined by comparison with the standard curve.

**Statistical Analysis.** The experimental design was a randomized complete block with three replications of treatments at all locations. Data were subjected to analysis of variance (ANOVA). Since a significant location interaction was present for yield and shikimic acid accumulation, data are presented by location and years. Since ANOVA established a significant difference in yield, injury, stunting, discoloration, and shikimic acid accumulation, a log-logistic function (equation 1) was used to describe the relationship between glyphosate rates and the respective variable (Seefeldt et al. 1995).

$$Y = A + B / (1 + \exp(D * (\log(\text{rate}) + \log(I_{50})))) \quad [1]$$

This nonlinear function is estimated by using A as the lower limit, B as the upper limit, I<sub>50</sub> is the concentration of glyphosate to cause 50% reduction, and D as the slope. Since this function utilizes the log of glyphosate rate, log rates of -2.35, -2.05, -1.75, -1.45, -1.15, -0.85, -0.55, -0.25, and 0.05 correspond to actual glyphosate rates of 0, 8.7, 17.4, 35, 70, 140, 280, 560, and 1,120 g/ha, respectively.

## Results

**Cotton Injury.** Visual injury was rated as a composite of percent stunting, discoloration, and stand reduction (Frans et al. 1986). Visual cotton injury, mainly discoloration and stunting at 7 DAT, increased at all locations with increasing glyphosate rates (Figure 1). Visible injury increased linearly at rates of 70

g/ha and higher (Figure 1). Glyphosate at 140 g/ha caused between 5 and 12% injury at 7 DAT (Figure 1). Discoloration was the earliest and most visible symptom of glyphosate injury. There were no reductions in cotton stand evaluations at 7 DAT.

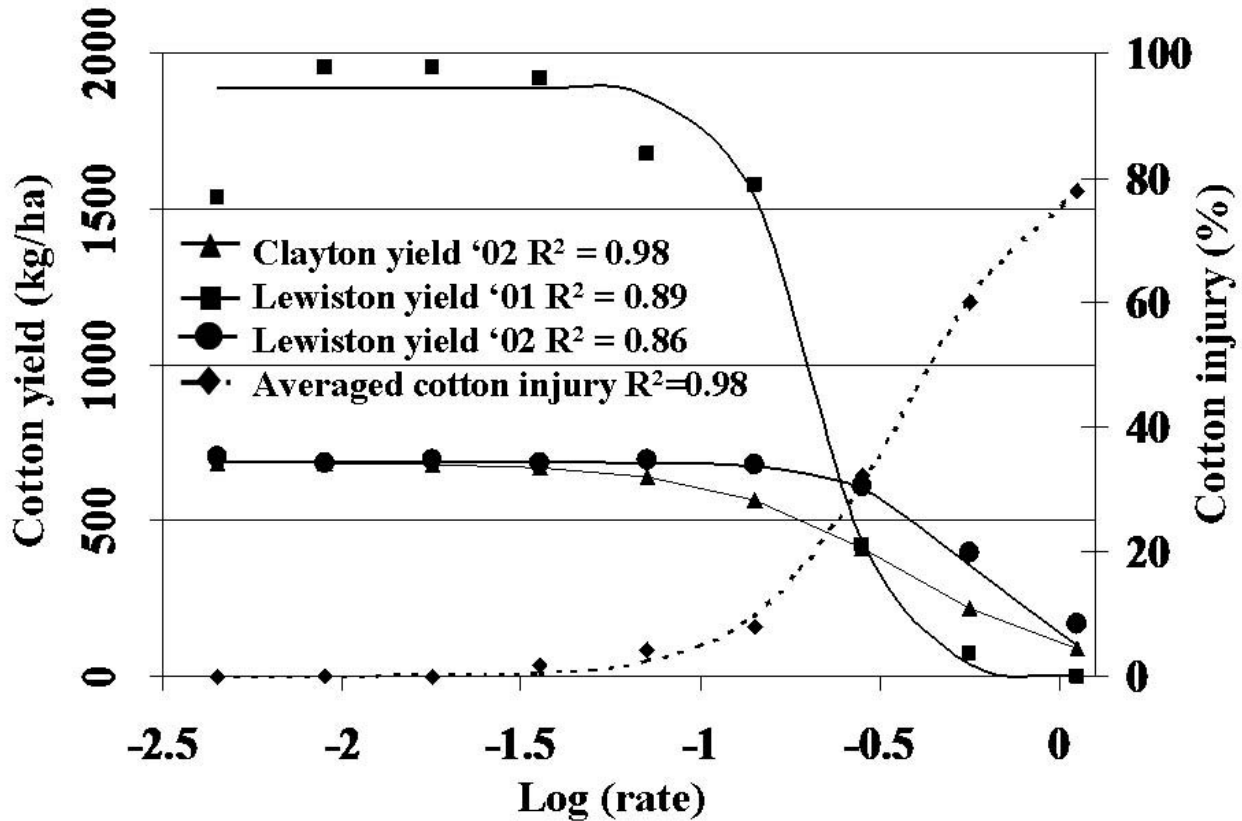
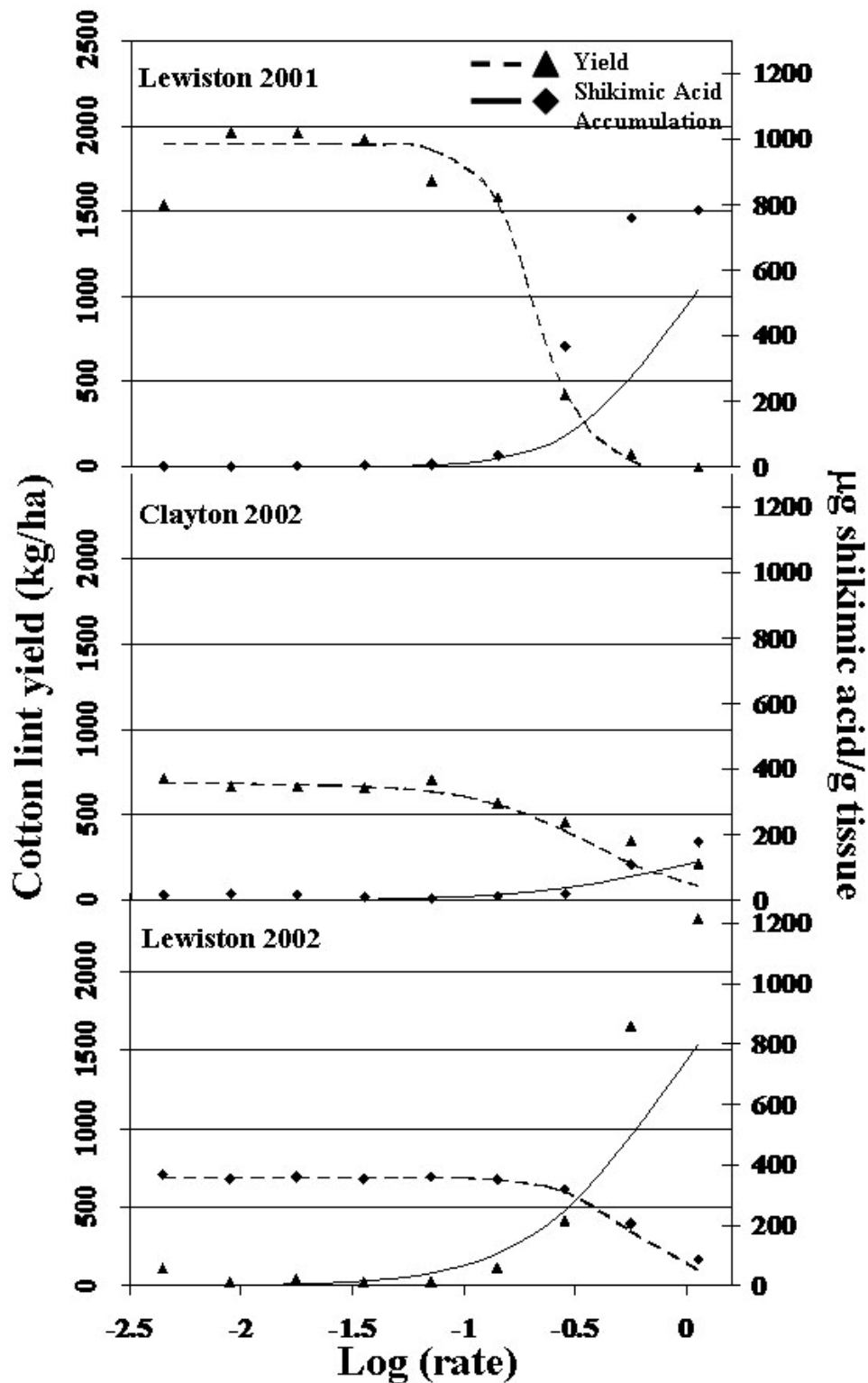


Figure 1. Relationship between yield and cotton visual injury at 7 DAT in response to simulated glyphosate drift, modeled using  $Y = A + B / (1 + \exp(D * (\log(\text{rate}) + \log(I50))))$ . Log rates of -2.35, -2.05, -1.75, -1.45, -1.15, -0.85, -0.55, -0.25, and 0.05 correspond to actual glyphosate rates of 0, 8.7, 17.4, 35, 70, 140, 280, 560, and 1,120 g/ha, respectively.

**Shikimic Acid Accumulation.** Shikimic acid accumulation is presented by location due to a significant treatment by location interaction. Plant tissue samples were taken at 7, 14, 21, and 28 DAT in 2001. Since no accumulation was found at 14, 21, and 28 DAT in 2001 (data not shown), samples were only taken 7 DAT in 2002. Shikimic acid accumulation increased with increasing glyphosate rates at all locations (Figure 2). Pline et al. (2002) found a similar increase in shikimic acid accumulation in the nontransgenic cotton variety DP 5415. At Lewiston 2001 and 2002, rates of 140 g/ha of glyphosate and greater caused shikimic acid accumulation (Figure 2). However, at the Clayton location, shikimic acid accumulation did not occur until 280 g/ha glyphosate. Accumulation in Clayton at 140 g/ha was 94 and 90% lower than in Lewiston 2001 and 2002, respectively (Figure 2). Lower shikimic acid accumulation in the Clayton trial may be due to the extreme drought situation during most of the growing season. No accumulation was observed with rates below 70 g/ha at any location.

**Yield.** Cotton lint yield data are presented by location due to significant treatment by location interactions. Nontreated cotton yielded 1720, 750, and 760 kg/ha of lint at Lewiston 2001 and 2002, and Clayton 2002, respectively (Figure 1). At Lewiston in 2001, yield of cotton treated with glyphosate at 8.7, 17.5, 35, and 70 g/ha was increased by 27, 27, 25, and 8%, respectively, compared to the nontreated



*Figure 2.* Relationship between yield and shikimic acid accumulation at 7 DAT in response to simulated glyphosate drift, modeled using  $Y = A + B/(1 + \exp(D * (\log(\text{rate}) + \log(I50))))$ . Log rates of -2.35, -2.05, -1.75, -1.45, -1.15, -0.85, -0.55, -0.25, and 0.05 correspond to actual glyphosate rates of 0, 8.7, 17.4, 35, 70, 140, 280, 560, and 1,120 g/ha, respectively.

check (Figure 1). Extremely low rates of herbicides including glyphosate, may have a stimulatory effect on plant growth (Banks and Schroeder 2002). Studies in 2002 did not show any increases in lint yield with 35 g/ha of glyphosate and lower (Figure 1). Cotton yields in 2001 were reduced when cotton was exposed to simulated glyphosate drift rates of 140 g/ha or higher (Figure 1). Locations in 2002 had similar levels of yield reductions, however, nontreated yields in 2002 were lower than 2001 yields due to the extreme drought situation.

### Conclusions

These results have shown that glyphosate drift rates of 140 g ai/ha and higher to nontransgenic cotton caused lint yield reductions and corresponding injury and shikimic acid accumulation depending on year and location. Although shikimic acid accumulation would predict potential yield reductions with glyphosate rates of 140 g/ha and higher, visual cotton was the most effective assay across years and locations. Since shikimic acid only accumulates in plants in response to glyphosate treatments, the two methods can be used collectively to conclude that glyphosate was the source of yield reductions.

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