

# Physiological basis for cotton tolerance to flumioxazin applied postemergence directed

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Previous research has shown that flumioxazin, a herbicide being developed as a postemergence-directed spray (PDS) in cotton, has the potential to injure cotton less than 30 cm tall if the herbicide contacts green stem tissue by rain splash or mis-application. In response to this concern, five-leaf cotton plants with chlorophyllous stems and older cotton, 16-leaf cotton plants, with bark on the lower stem were treated with a PDS containing flumioxazin plus crop oil concentrate (COC) or nonionic surfactant (NIS). Stems of treated plants and untreated plants at the respective growth stage were cross-sectioned and then magnified and photographed using bright-field microscopy techniques. More visible injury consisting of necrosis and desiccation was evident in younger cotton. Also, there was a decrease in treated-stem diameter and an increase in visible injury with COC compared with NIS in younger cotton. The effects of plant growth stage and harvest time on absorption, translocation, and metabolism of  $^{14}\text{C}$ -flumioxazin in cotton were also investigated. Total  $^{14}\text{C}$  absorbed at 72 h after treatment (HAT) was 77, 76, and 94% of applied at 4-, 8-, and 12-leaf growth stages, respectively. Cotton at the 12-leaf stage absorbed more  $^{14}\text{C}$  within 48 HAT than was absorbed by four- or eight-leaf cotton by 72 HAT. A majority (31 to 57%) of applied  $^{14}\text{C}$  remained in the treated stem for all growth stages and harvest times. Treated cotton stems at all growth stages and harvest times contained higher concentrations ( $\text{Bq g}^{-1}$ ) of  $^{14}\text{C}$  than any other tissues. Flumioxazin metabolites made up less than 5% of the radioactivity found in the treated stem. Because of the undetectable levels of metabolites in other tissues when flumioxazin was applied PDS, flumioxazin was foliar applied to determine whether flumioxazin transported to the leaves may have been metabolized. In foliar-treated cotton, flumioxazin metabolites in the treated leaf of four-leaf cotton totaled 4% of the recovered  $^{14}\text{C}$  72 HAT. Flumioxazin metabolites in the treated leaf of 12-leaf cotton totaled 35% of the recovered  $^{14}\text{C}$  48 HAT. These data suggest that differential absorption, translocation, and metabolism at various growth stages, as well as the development of a bark layer, are the bases for differential tolerances of cotton to flumioxazin applied PDS.

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

**Key words:** Absorption, translocation, metabolism.

Flumioxazin was recently registered for preemergence use in peanut (*Arachis hypogaea* L.) as well as for a preplant fall or spring burndown treatment in cotton, and it is also being developed as a postemergence-directed spray (PDS) treatment in cotton (Anonymous 2002; Askew et al. 2002; Price and Wilcut 2002). Flumioxazin acts by inhibiting protoporphyrinogen oxidase (protoporphyrin IX:oxygen oxidoreductase, EC 1.3.3.4) (Anonymous 1988; Cranmer et al. 2000). Inhibition of this enzyme induces accumulation of protoporphyrin IX because of uncontrolled auto-oxidation of the substrate (Duke et al. 1991). As protoporphyrin IX accumulates and is impinged by light, toxic oxygen radicals are generated, which lead to degradation of plasmalemma and tonoplast membrane lipids and irreversible damage of their membrane function and structure in susceptible plants.

Acifluorfen, lactofen, and sulfentrazone are herbicides that have similar modes of action as flumioxazin and are registered in soybean [*Glycine max* (L.) Merr.]. Plant species differ in their tolerance to these herbicides because of differential absorption, translocation, and metabolism. Foliar-applied  $^{14}\text{C}$ -acifluorfen was absorbed slowly by tolerant soybean leaves, with only 4% absorbed 48 h after treatment

(HAT) compared with 11% by both susceptible common ragweed (*Ambrosia artemisiifolia* L.) and common cocklebur (*Xanthium strumarium* L.) (Ritter and Coble 1981). In a similar study, tolerant ivyleaf morningglory (*Ipomoea hederacea* L.) absorbed 29% of applied  $^{14}\text{C}$ -acifluorfen and 36% of applied  $^{14}\text{C}$ -lactofen, whereas susceptible or sensitive pitted morningglory (*Ipomoea lacunosa* L.) absorbed 68% of applied  $^{14}\text{C}$ -acifluorfen and 36% of applied  $^{14}\text{C}$ -lactofen, with less than 1% of applied herbicide translocated out of the treated leaf in either morningglory species (Higgins et al. 1988). Comparing sicklepod [*Senna obtusifolia* L. (Irwin and Barnaby)] and coffee senna (*Cassia occidentalis* L.) susceptibility to sulfentrazone, Dayan et al. (1996) found that sicklepod, which exhibits considerable tolerance, metabolized 92% of applied sulfentrazone at 9 HAT. Coffee senna, a sensitive species, absorbed 74% more sulfentrazone than sicklepod and metabolized only 17% by 9 HAT. In another study, Dayan et al. (1997) reported no differences in absorption and translocation of sulfentrazone between relatively tolerant and less tolerant soybean cultivars. Tolerance was due to differential metabolism and differential tolerance to herbicide-induced peroxidative stress tolerance.



FIGURE 1. Cross section of untreated five-leaf cotton stem ( $\times 1.67$ ).

Localized injury has been observed on chlorophyllous green cotton stems less than 30 cm tall when flumioxazin was applied as a PDS (Altom et al. 2000). Severe injury may occur when flumioxazin contacts cotton foliage as when heavy rain splashes treated soil onto leaves or when the herbicide is misapplied (Wilcut et al. 2000). Flumioxazin can be applied safely as a precise PDS to 15- to 30-cm-tall cotton (Askew et al. 2002; Main et al. 2000). Less injury is

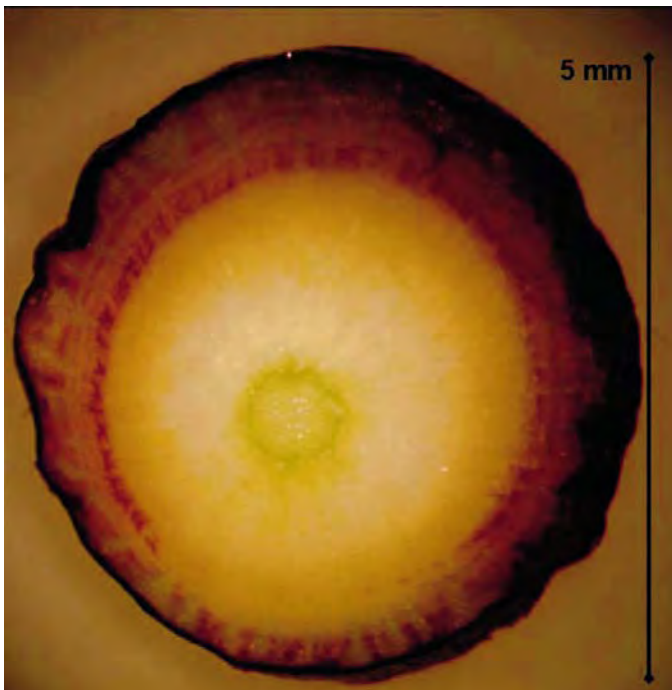


FIGURE 2. Cross section of treated zone from five-leaf cotton stem treated with flumioxazin applied as a PDS at  $0.071 \text{ kg ai ha}^{-1}$  plus  $0.25\% \text{ v/v NIS}$  ( $\times 3.5$ ).

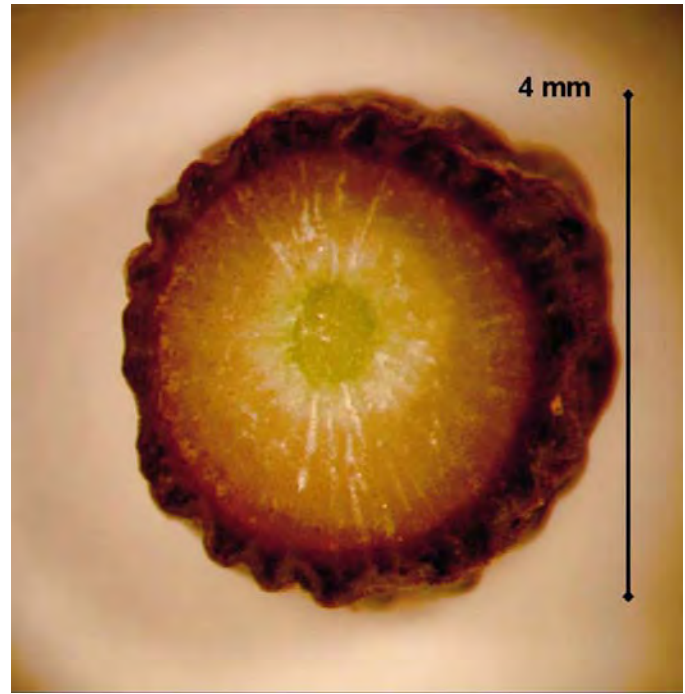


FIGURE 3. Cross section of treated zone from five-leaf cotton stem treated with flumioxazin applied as a PDS at  $0.071 \text{ kg ai ha}^{-1}$  plus  $1.0\% \text{ v/v COC}$  ( $\times 3.5$ ).

observed on older cotton plants (approximately 12-leaf stage), in which a layer of bark develops on the stem up to approximately 10 cm above the soil surface. This increased tolerance may be due to decreased flumioxazin absorption into the stem, increased translocation out of the stem, or increased metabolism by more mature plants. The presence of a bark layer composed of highly lignified cells may also minimize localized flumioxazin injury. The objectives of this research were to evaluate the absorption, translocation, and metabolism of  $^{14}\text{C}$ -flumioxazin as well as herbicidal damage to stem tissue in cotton as influenced by growth stage and

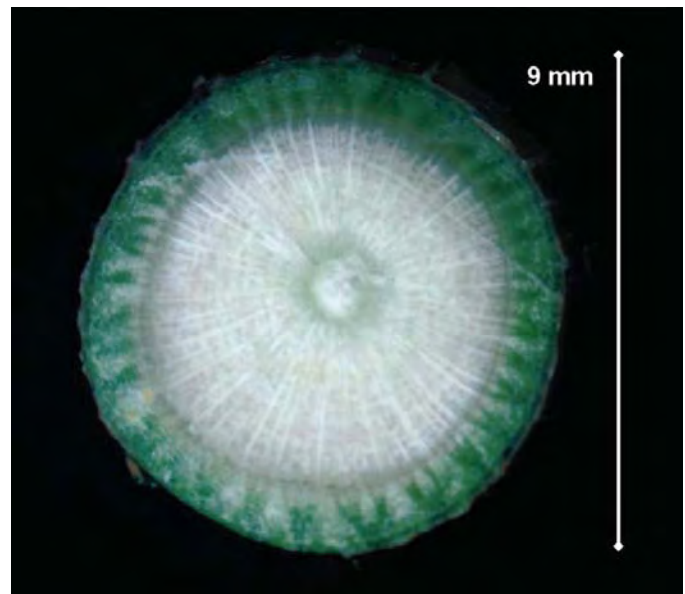


FIGURE 4. Cross section of untreated 16-leaf cotton stem ( $\times 1.67$ ).

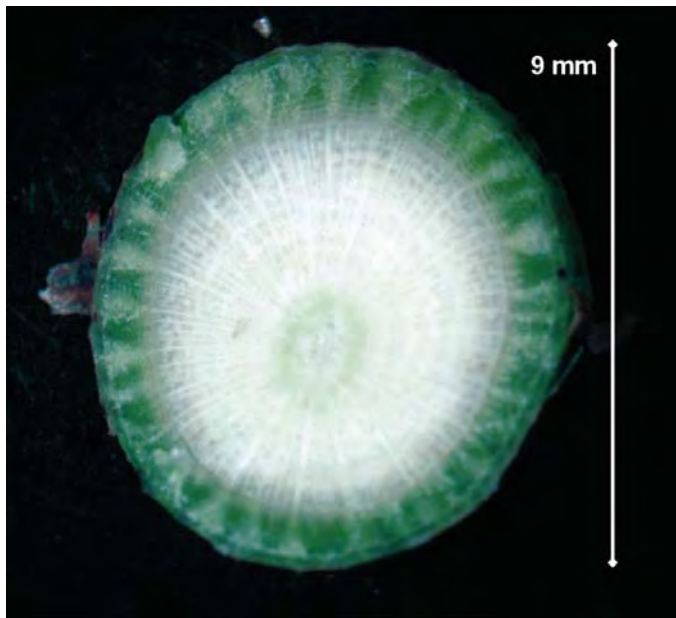


FIGURE 5. Cross section of treated zone from 16-leaf cotton stem treated with flumioxazin applied as a PDS at  $0.071 \text{ kg ai ha}^{-1}$  plus  $0.25\% \text{ v/v}$  NIS ( $\times 3.5$ ).

harvest times to explain increased tolerance of mature cotton vs. younger cotton to flumioxazin applied as a PDS.

## Materials and Methods

### Plant Material and Growth Conditions

Cotton plants ('DeltaPine 5415RR') were grown in 15-L pots containing Metro-Mix 360<sup>1</sup> in a plastic greenhouse maintained at approximately  $25 \pm 2 \text{ C}$ . There was a 16-h photoperiod of natural and supplemental metal halide lighting with an average midday photosynthetic flux density of  $700 \mu\text{mol m}^{-2} \text{ s}^{-1}$ . Four seeds were sown, and seedlings were thinned to one plant per pot at the two-leaf stage. Studies were arranged as a randomized complete block with three replications of treatments to evaluate absorption and translocation of flumioxazin. Each study was repeated in time. Two separate studies were conducted to evaluate the metabolism of flumioxazin. These studies were also repeated in time.

### Microscopy

To visually assess the type and tissue depth of flumioxazin injury to five-leaf cotton plants with chlorophyllous stems as well as to 16-leaf cotton plants with mature bark, stems were treated with a PDS containing  $0.071 \text{ kg ai ha}^{-1}$  flumioxazin plus  $1.0\% \text{ (v/v)}$  crop oil concentrate<sup>2</sup> (COC) or  $0.25\% \text{ (v/v)}$  nonionic surfactant<sup>3</sup> (NIS) using a backpack sprayer delivering an output of  $140 \text{ L ha}^{-1}$  through a hooded boom equipped with 1102VS flat-fan nozzles. Flumioxazin application was made to a height of 10 cm on the cotton stem above the soil surface. Stems of treated and untreated cotton plants were cross-sectioned by hand using a razor blade at 5 cm above the soil surface 9 d after treatment. Each treatment was replicated three times. Stem sections were observed under a dissecting microscope and photographed with a digital camera. Photographs show the av-

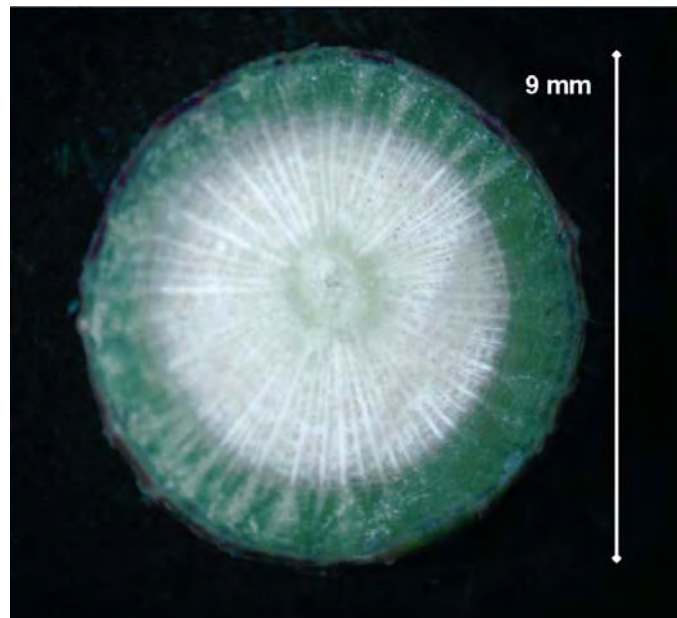


FIGURE 6. Cross section of treated zone from 16-leaf cotton stem treated with flumioxazin applied as a PDS at  $0.071 \text{ kg ai ha}^{-1}$  plus  $1.0\% \text{ v/v}$  COC ( $\times 3.5$ ).

erage injury consisting of necrosis and desiccation present at 5 cm above soil surface for each treatment.

### Absorption and Translocation

Plants at the 4-, 8-, and 12-leaf growth stages were treated with [phenyl-<sup>14</sup>C] flumioxazin. A microsyringe was used to deliver ten  $1\text{-}\mu\text{l}$  droplets of <sup>14</sup>C-flumioxazin<sup>4</sup> plus  $0.25\% \text{ (v/v)}$  NIS in water containing  $1,250 \text{ Bq } ^{14}\text{C-flumioxazin}$  ( $106 \text{ mCi/mmol}$  specific activity,  $94.2\%$  radiopurity) to a  $5\text{-cm}^2$  section of stem just above soil level to simulate a precise PDS application. Treated plants were harvested 4, 24, 48, or 72 HAT. At harvest, the treated stem from each plant was washed with 10 ml of methanol–water ( $1:1, \text{ v/v}$ ) and  $0.25\% \text{ (v/v)}$  NIS solution to remove nonabsorbed flumioxazin from the stem surface. A 1-ml aliquot from each stem wash was then added to 25 ml of scintillation cocktail<sup>5</sup> and quantified by liquid scintillation spectroscopy<sup>6</sup> (LSS). The remainder of the plant was then sectioned into treated and untreated portions: the stem, mature fully expanded leaves, immature leaves and buds, roots, and, when applicable, reproductive squares and bolls. These parts were then placed in paper bags and dried at  $65 \text{ C}$  for at least 72 h. The plant parts were then ground with a coffee grinder,<sup>7</sup> and a 100- to 200-mg subsample was oxidized in a biological oxidizer<sup>8</sup>, where <sup>14</sup>C was trapped in scintillation cocktail and radioactivity quantified by LSS.

Data were subjected to analysis of variance (ANOVA) using SAS<sup>9</sup>. Residuals were plotted, and logarithmic transformations were conducted on data, where variance increased with increasing means. After ANOVA, treatment or log-transformed treatment means were compared using Fisher's Protected LSD test at the 5% probability level.

### Metabolism

To evaluate whether growth stage influenced metabolism, two separate studies were conducted. In the first study, cot-



TABLE 1. Distribution of  $^{14}\text{C}$  among plant parts and over time from  $^{14}\text{C}$ -flumioxazin applied to the lower stem of four-leaf cotton.<sup>a</sup>

Tissue	4 HAT	24 HAT	48 HAT	72 HAT
Bq g <sup>-1</sup> (% of total applied)				
Treated stem	7,200 a <sup>b</sup> (33)	7,343 a(41)	9,095 a(53)	6,344 a(56)
Mature leaf	26 h(1)	55 fgh(4)	103 def(7)	39 h(11)
Immature leaves/buds	45 h(1)	75 gef(1)	1,048 bc(3)	389 def(3)
Untreated stem	137 cde(2)	190 bcde(2)	458 b(6)	254 bcd(6)
Roots	178 cde(2)	151 cde(1)	83 efg(1)	43 gh(1)
Fruiting branches	NA	NA	NA	NA
Squares	NA	NA	NA	NA
Nonabsorbed	NA(61)	NA(51)	NA(30)	NA(23)

<sup>a</sup> Abbreviations: HAT, hours after treatment; NA, not applicable.

<sup>b</sup> Leaf stage was separated using Fisher's Protected LSD on log-transformed data. Means followed by the same letter are not significantly different ( $P = 0.05$ ).

ton plants were grown and treated with 1,250 Bq  $^{14}\text{C}$ -flumioxazin on the treated stem (4-, 8-, and 12-leaf cotton). Because of the undetectable levels of metabolites in tissue other than the treated stem when flumioxazin was applied PDS, flumioxazin was then foliar applied in the second study to determine whether flumioxazin transported to the leaves could be metabolized.  $^{14}\text{C}$ -flumioxazin containing 5,830 Bq was spotted on the youngest fully expanded leaf (4- and 12-leaf cotton) and harvested as previously described. At harvest, partitioned plant parts were immediately placed in a freezer at  $-30\text{ C}$  until further analysis. Based on absorption and translocation data from the previous study, we assumed only treated stems and treated leaves contained sufficient amounts of  $^{14}\text{C}$  for detection of metabolites.

Stems or leaves were ground into a fine powder with a mortar and pestle under liquid nitrogen. The plant material was then placed in a 20-ml centrifuge vial, and 5 ml of acetone was added to extract  $^{14}\text{C}$ -flumioxazin and possible metabolites. The samples were vortexed for 1 min and were allowed to extract for 2 h. The tissue samples were then centrifuged for 15 min at  $2,000 \times g$ . The supernatant was decanted and filtered for each sample. The tissue was re-extracted twice as above, and the resulting supernatant was combined and evaporated to a consistent volume of 300  $\mu\text{l}$ . The remaining extracted stem or leaf material was oxidized and nonextracted  $^{14}\text{C}$  quantified as previously described. The supernatant was analyzed using Waters® high-performance liquid chromatography (HPLC) instrumentation<sup>10</sup> to separate the parent herbicide from possible metabolites, which were detected and quantified using a Waters® UV spectrophotometer<sup>10</sup> and a Packard® in-line radioactive detector.<sup>11</sup> Radioactive trace peaks were integrated with FLOW-ONE® software,<sup>11</sup> with background excluded from peak area calculations. Comparing retention times from a  $^{14}\text{C}$ -flumioxazin standard and retention times within samples identified the nonmetabolized  $^{14}\text{C}$ -flumioxazin parent.

The HPLC conditions were a modification of those supplied by Valent USA. The initial mobile phase was acetonitrile plus 0.01% trifluoroacetic acid 25:75 (v/v), followed by a linear increase to 100% acetonitrile from 0 to 20 min. The 100% acetonitrile mobile phase was then held constant until 30 min after sample injection. The mobile phase flow rate was 1 ml min<sup>-1</sup>. The flow rate for scintillation cocktail to the radioactive detector was 3 ml min<sup>-1</sup>. A reverse-phase C-18 column<sup>12</sup> (25- by 4.6-mm column dimensions, 10- $\mu\text{m}$  particle size) was used for the separation. All solvents

were of HPLC grade, and all injection volumes were 25  $\mu\text{l}$ . Statistical procedures were similar to those previously described for absorption and translocation data.

## Results and Discussion

### Visible Injury by Flumioxazin

Visible injury consisting of necrosis and desiccation to cotton stems caused by flumioxazin was more severe in younger (five-leaf stage) cotton than in older (16-leaf) cotton (Figures 1–6). The adjuvant added to flumioxazin also affected the severity of injury to cotton stem tissue. Treatments containing COC plus flumioxazin decreased treated-stem diameter and increased the depth of flumioxazin injury in stems of five-leaf cotton compared with the untreated and NIS plus flumioxazin (Figures 1–3). Visible injury and decreases in stem diameter were not evident in any flumioxazin treatment to 16-leaf cotton regardless of surfactant (Figures 4–6). These micrographs demonstrate that flumioxazin treatments were more injurious to five-leaf cotton than to 16-leaf cotton.

### Absorption and Translocation

Growth stage by trial and harvest timing by trial interactions were not significant ( $P > 0.05$ ), thus data were combined over experimental trial. The effects of growth stage and harvest time significantly influenced the absorption and translocation of  $^{14}\text{C}$  by cotton. At 4 HAT, total absorption of  $^{14}\text{C}$ -flumioxazin was 39, 40, and 60% for 4-, 8-, and 12-leaf growth stages, respectively (Tables 1–3). Total  $^{14}\text{C}$  absorbed by 72 HAT was 78, 76, and 95% at 4-, 8-, and 12-leaf growth stages, respectively. Overall, absorption of  $^{14}\text{C}$ -flumioxazin in the current study was higher than previously reported by Higgins et al. (1988) and Ritter and Coble (1981) for herbicides (lactofen and acifluorfen, respectively) with a similar mode of action. These differences may reflect differences in our application to the lower cotton stem vs. foliar application to soybean. Wills (1978) found that translocation of  $^{14}\text{C}$ -glyphosate was greater after application to the mature lower stem than when applied to mature lower leaves or to immature upper stem or leaves of cotton.  $^{14}\text{C}$  absorption by 12-leaf cotton within 24 HAT exceeded that by four- or eight-leaf cotton at 72 HAT (Figure 7). Despite greater  $^{14}\text{C}$  absorption by 12-leaf cotton, visible injury at this stage is far less than that observed in younger cotton

TABLE 2. Distribution of  $^{14}\text{C}$  among plant parts and over time from  $^{14}\text{C}$ -flumioxazin applied to the lower stem of eight-leaf cotton.<sup>a</sup>

Tissue	4 HAT	24 HAT	48 HAT	72 HAT
Bq g <sup>-1</sup> (% of total applied)				
Treated stem	3,445 a <sup>b</sup> (31)	5,351 a(47)	3,542 a(54)	6,801 a(57)
Mature leaf	402 de(3)	1,180 b(8)	967 bc(7)	775 bcd(6)
Immature leaves/buds	252 e(2)	153 f(1)	459 de(3)	364 de(3)
Untreated stem	299 e(2)	401 de(3)	463 cde(3)	871 bc(6)
Roots	296 e(2)	736 bce(5)	472 cde(4)	514 cde(4)
Fruiting branches	NA	NA	NA	NA
Squares	NA	NA	NA	NA
Nonabsorbed	NA(60)	NA(36)	NA(29)	NA(24)

<sup>a</sup> Abbreviations: HAT, hours after treatment; NA, not applicable.

<sup>b</sup> Leaf stage was separated using Fisher's Protected LSD on log-transformed data. Means followed by the same letter are not significantly different ( $P = 0.05$ ).

(personal observation). Pline et al. (2001) reported greater absorption of stem-applied  $^{14}\text{C}$ -glyphosate by 12-leaf cotton than by four- or eight-leaf cotton. These studies, as well as the current study, suggest that herbicide entry through the bark and cambium tissue layers of the mature cotton stem is greater than through other tissues. In the case of flumioxazin, this tissue may be less sensitive to injury than foliar or green stem tissue because of lower protoporphyrin IX content in this lignified tissue (Duke et al. 1974–1994). A lack of stem tissue damage by flumioxazin may allow continued absorption of the herbicide over time.

The majority (31 to 57%) of applied  $^{14}\text{C}$  remained in the treated stem for all growth stages and harvest times. Only 1 to 2% of  $^{14}\text{C}$  was translocated to mature leaves, immature leaves and buds, untreated stem, and roots of four-leaf cotton by 4 HAT. However, translocation increased with time in four-leaf cotton to 11% in mature leaves, 3% in immature leaves and buds, and 6% in untreated stem but remained at only 1% in roots at 72 HAT. Translocation of absorbed  $^{14}\text{C}$  to mature leaves was greater in eight-leaf cotton than at the four-leaf stage at 4 HAT, but only 2% or less  $^{14}\text{C}$  was translocated to immature leaves and buds, roots, and the untreated stem portion (Table 1). Translocation of absorbed  $^{14}\text{C}$  over time in eight-leaf cotton increased to 6% in mature leaves and in untreated stem but did not increase in immature leaves and buds or roots by 72 HAT. This increase was similar to that in four-leaf cotton. Roots of 12-leaf cotton contained at least three times more  $^{14}\text{C}$  than roots of four- and eight-leaf cotton at all harvest times.

Treated cotton stems at all growth stages and harvest

times contained greater concentrations (Bq g<sup>-1</sup>) of  $^{14}\text{C}$  than any other tissues (Tables 1–3). At the four-leaf stage, the concentration of  $^{14}\text{C}$  in all other tissues remained below 10% of the concentration detected in the treated stem (Table 1). The injury associated with flumioxazin to green stems at this stage may limit the amount of  $^{14}\text{C}$  translocated from the treated stem.  $^{14}\text{C}$ -flumioxazin applied to 8- and 12-leaf cotton was more likely to translocate from the treated stem and accumulate in other tissues.  $^{14}\text{C}$  applied to eight-leaf cotton initially accumulated in the mature leaf tissue (4 to 48 HAT) but later began to accumulate in stem tissue (72 HAT) (Table 2).  $^{14}\text{C}$ -flumioxazin applied to 12-leaf cotton tended to accumulate in roots (Table 3). As tissues increase in mass from the four- to the 12-leaf stages, one would expect the concentration of  $^{14}\text{C}$  to decrease as the herbicide is diluted by the increase in biomass. This decrease was observed with the treated stem tissue; however,  $^{14}\text{C}$  concentration increased in most other tissues from the four- to eight-leaf stage despite the increase in biomass. The increase in  $^{14}\text{C}$  concentration in other tissues would suggest that considerably more translocation was occurring in 8- and 12-leaf cotton than in four-leaf cotton, potentially diluting the concentration in treated stems to a less injurious concentration. In 12-leaf cotton, <5% of absorbed  $^{14}\text{C}$  translocated to reproductive structures. However, on a Bq g<sup>-1</sup> tissue dry weight concentration basis, this represents at least one-tenth and up to one-third the concentration of  $^{14}\text{C}$  detected in treated stems. Whether this concentration would affect normal fruit development is not known. However, lint yield was not influenced by flumioxazin treatment in field studies (Askew et al. 2002).

TABLE 3. Distribution of  $^{14}\text{C}$  among plant parts and over time from  $^{14}\text{C}$ -flumioxazin applied to the lower stem of 12-leaf cotton.<sup>a</sup>

Tissue	4 HAT	24 HAT	48 HAT	72 HAT
Bq g <sup>-1</sup> (% of total applied)				
Treated stem	1,124 hijk <sup>b</sup> (33)	3,834 abc(48)	3,029 ab(52)	5,049 a(53)
Mature leaf	398 defghi(6)	554 def(7)	1,650 de(8)	538 def(7)
Immature leaves/buds	146 ijkl(1)	110 jkl(1)	83 kl(1)	326 fghij(3)
Untreated stem	580 defg(6)	377 defgh(4)	333 defghi(4)	907 de(11)
Roots	585 hijk(10)	1,060 de(13)	1,454 cd(22)	1,146 bcd(15)
Fruiting branches	185 ghijk(3)	296 efghij(4)	454 defghi(5)	339 defghi(4)
Squares	181 defg(1)	63 kl(1)	44 defghi(1)	682 hijk(1)
Nonabsorbed	NA(40)	NA(22)	NA(7)	NA(6)

<sup>a</sup> Abbreviations: HAT, hours after treatment; NA, not applicable.

<sup>b</sup> Leaf stage was separated using Fisher's Protected LSD on log-transformed data. Means followed by the same letter are not significantly different ( $P = 0.05$ ).

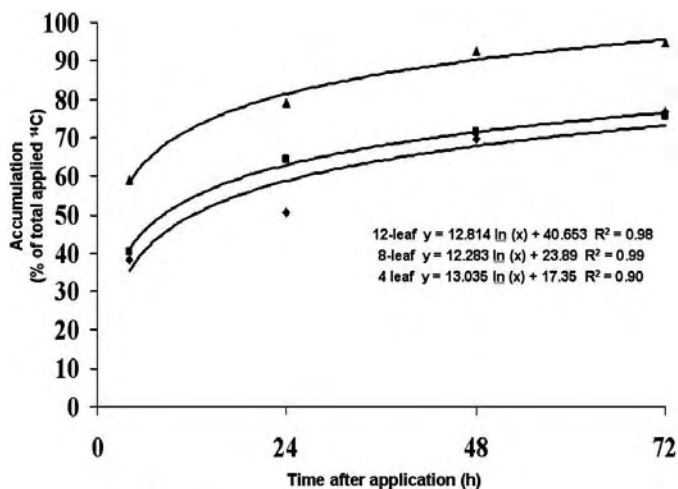


FIGURE 7. Total accumulation of  $^{14}\text{C}$  from stem-applied  $^{14}\text{C}$ -flumioxazin in 4-leaf (◆), 8-leaf (■), and 12-leaf (▲) cotton.

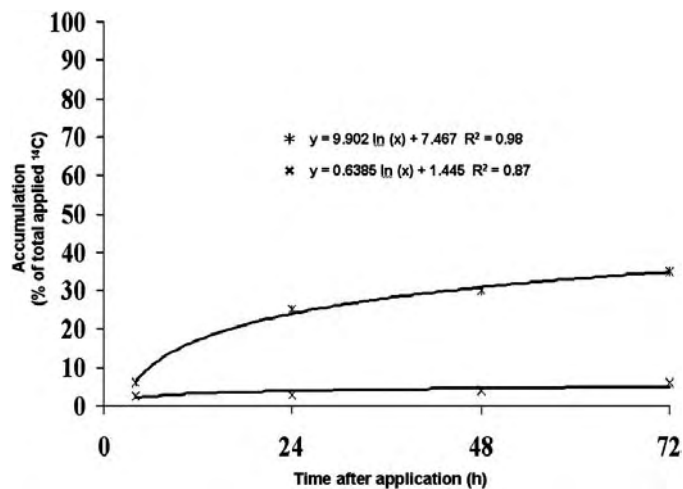


FIGURE 8. Total accumulation of possible flumioxazin metabolites in 4-leaf (x) and 12-leaf (\*) cotton from foliar-applied  $^{14}\text{C}$ -flumioxazin.

## Metabolism

Experimental trial by main effect interactions were not significant for the two studies ( $P > 0.05$ ), so data were combined over trial. Harvest time significantly influenced metabolism of foliar-applied  $^{14}\text{C}$ -flumioxazin but not stem-applied  $^{14}\text{C}$ -flumioxazin. Extraction of absorbed radioactivity was greater than 90%. Of the recovered radioactivity, 10% remained bound in the plant debris after extraction. The parent flumioxazin eluted at 22.5 min. Other  $^{14}\text{C}$  peaks assumed to be metabolites eluted between 15.1 and 23.3 min. Thus, the amount of herbicide metabolites in the stem or leaves was the sum of these metabolites.

Flumioxazin metabolites recovered in the stem totaled less than 0.05% of total applied  $^{14}\text{C}$  and around 5% of the recovered  $^{14}\text{C}$  found in the treated stem (data not shown). Accumulation of metabolites in treated stem portions of 4-, 8-, and 12-leaf stages at 4, 24, 48, and 72 HAT was not significantly different (data not shown). The main effects of growth stage as well as harvest timing were significant for metabolites recovered in foliar-applied treated leaves. Flumioxazin metabolites in the treated leaf of four-leaf cotton totaled 4% of the applied  $^{14}\text{C}$  72 HAT (Figure 8). Flumioxazin metabolites in the treated leaf of 12-leaf cotton totaled 35% of the applied  $^{14}\text{C}$  72 HAT. All metabolites detected were more polar than flumioxazin. Because stem-applied flumioxazin is transported to leaves (7% at 72 HAT), these data suggest that it could be metabolized within the leaf. Because the purity of the applied  $^{14}\text{C}$ -flumioxazin was 94.2%, it is possible that the metabolites detected were present as contaminants within the applied solution; however, samples spiked with  $^{14}\text{C}$ -flumioxazin did not result in metabolites having increased peak area (data not shown).

In summary, these data suggest that differential absorption and translocation at various growth stages and, to a lesser extent, increased metabolism by more mature cotton plants contribute to differential tolerance of cotton receiving flumioxazin as a PDS at different growth stages. Differential absorption, not translocation or metabolism, was found to be the basis for soybean tolerance to root-absorbed sulfentrazone during the earliest stages of development (Li et al. 2000). However, postemergence-applied acifluorfen tolerance in eastern black nightshade (*Solanum ptycanthum* L.)

has been shown to be due to differential metabolism compared with susceptible somaclones (Yu and Masiunas 1992). In cases where injury is observed on young cotton chlorophyllous green stem tissue after flumioxazin is applied as a PDS, injury likely results from localized high concentrations of flumioxazin on the treated stem area as a result of lower levels of translocation out of the stem region compared with older cotton and also because chlorophyllous tissue is where flumioxazin-induced peroxidation could be expressed. Further, in older cotton plants with a bark layer on the lower stem, less localized injury was observed because of woody outer layers of bark tissue and more rapid translocation of herbicide out of the treated stem area to areas where it may be metabolized. Also, lower concentrations in the treated stem due to greater biomass of an older cotton plant, continued absorption of applied flumioxazin into the stem from the treated stem surface, and limited subsequent translocation and potential metabolism may dilute flumioxazin concentration at the treated stem surface. These cumulative factors may contribute to reducing observed localized injury in older cotton.

## Sources of Materials

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

<sup>2</sup> Agridex®, 83% paraffin base petroleum oil and 17% surfactant blend, Helena Chemical Co., 7664 Moore Road, Memphis, TN 38120.

<sup>3</sup> Induce® nonionic low-foam wetter-spreader adjuvant contains 90% nonionic surfactant (alkylaryl polyoxyalkane ether and isopropanol), free fatty acids, and 10% water, Helena Chemical Co., 7664 Moore Road, Memphis, TN 38120.

<sup>4</sup> Valent USA Corporation, P.O. Box 8025, Walnut Creek, CA 94596-8025.

<sup>5</sup> ScintiVerse® SX18-4 Universal Liquid Scintillation Cocktail, Fisher Scientific, Fairlawn, NJ 07410.

<sup>6</sup> Packard TRI-CARB 2100TR Liquid Scintillation Spectrometer, Packard Instrument Company, 2200 Warrensville Road, Downers Grove, IL 60515.

<sup>7</sup> Coffee Mill, Mr. Coffee, 24700 Miles Road, Bedford Heights, OH 44146-1399.

<sup>8</sup> Harvey biological oxidizer, J. Harvey Instrument Corporation, 123 Patterson Street, Hillsdale, NJ 07642.

<sup>9</sup> Statistical Analysis Systems (SAS) software, Version 8, SAS Institute Inc., Box 8000, SAS Circle, Cary, NC 27513.

<sup>10</sup> Waters Corporation, 34 Maple Street, Milford, MA 01757.

<sup>11</sup> Packard Instrument Company, 800 Research Parkway, Meridian, CT 06450.

<sup>12</sup> Alltech Associates, Inc., 2051 Waukegan Road, Deerfield, IL 60015.

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