

Absorption, translocation, and metabolism of foliar-applied CGA-362622 in purple and yellow nutsedge (*Cyperus rotundus* and *C. esculentus*)

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Studies were conducted to evaluate the absorption, translocation, and metabolism of ^{14}C -CGA-362622 when foliar-applied to purple and yellow nutsedge. Less than 53% of the herbicide was absorbed after 96 h. Both nutsedge species translocated appreciable amounts of herbicide (30%) out of treated leaves. Translocation was both acropetal and basipetal, with at least 25% transported basipetally. Neither nutsedge species translocated more than 4% of applied radioactivity to the tubers and roots. Most of the metabolites formed by the nutsedge species were more polar than ^{14}C -CGA-362622 and averaged 69 and 61% of the radioactivity in purple and yellow nutsedge, respectively. The half-life of CGA-362622 was estimated at 4 h in both purple and yellow nutsedge.

Nomenclature: CGA-362622, *N*-([4,6-dimethoxy-2-pyrimidinyl]carbamoyl)-3-(2,2,2-trifluoroethoxy)-pyridin-2-sulfonamide sodium salt; purple nutsedge, *Cyperus rotundus* L. CYPRO; yellow nutsedge, *Cyperus esculentus* L. CYPES.

Key words: Acropetal translocation, basipetal translocation, half-life.

Purple and yellow nutsedge are herbaceous perennial weeds that are among the world's worst pests (Stoller and Sweet 1987). Holm et al. (1977) listed purple nutsedge as the world's worst weed and yellow nutsedge as the 16th worst weed. *Cyperus* species are especially problematic in the southeastern United States, primarily because of their perennial nature, longevity of tubers, and prolific tuber production (Bariuan et al. 1999). Successful control of *Cyperus* species with foliar-applied herbicides requires rapid absorption and translocation to the meristematic regions of the basal bulb, rhizomes, and tubers (Sprankle et al. 1975). But increased use of herbicides that inadequately control *Cyperus* species has created a niche for these weeds by eliminating interspecific weed competition (Bendixen and Stroube 1977).

Purple and yellow nutsedge are among the 10 most troublesome weeds in cotton (*Gossypium hirsutum* L.) (Dowler 1998). In the past, cotton growers have relied on a preplant-incorporated treatment of norflurazon, followed by either DSMA or MSMA postemergence-directed, followed by several cultivations for acceptable nutsedge control (McLean et al. 2001; Wilcut et al. 1995; York 1994). But recent herbicide developments have expanded the postemergence options for selective control of nutsedge in cotton. Currently, pyriithiobac is the only selective, postemergence-applied herbicide registered for use in nontransgenic cotton that offers some control of purple and yellow nutsedge (Wilcut 1998).

CGA-362622 is a new sulfonylurea herbicide being developed for postemergence weed control in nontransgenic and transgenic cotton and sugarcane (*Saccharum* spp.) (Holloway et al. 2000; Hudetz et al. 2000; Porterfield et al. 2002a, 2002b; Wells et al. 2000). CGA-362622, as a sulfonylurea herbicide, inhibits acetolactate synthase (ALS) (EC 4.1.3.18) in susceptible plants (Hudetz et al. 2000).

Cotton tolerance is due to limited absorption and rapid metabolism (Askew and Wilcut 2002). CGA-362622 controls a broad spectrum of broadleaf weeds in cotton (Holloway et al. 2000; Hudetz et al. 2000; Porterfield et al. 2002a). In addition, control of purple and yellow nutsedge has been reported in greenhouse and field experiments (Brecke et al. 2000; Holloway et al. 2000; Porterfield et al. 2002a; Troxler et al. 2002). Porterfield et al. (2002a) observed that CGA-362622 controlled yellow nutsedge better than did pyriithiobac.

Several ALS-inhibiting herbicides control purple and yellow nutsedge, including chlorimuron (Reddy and Bendixen 1988), imazapic (Richburg et al. 1994), imazaquin (Nandihalli and Bendixen 1988), imazethapyr (Richburg et al. 1993), and pyriithiobac (Wilcut 1998). Tolerance to sulfonylurea and imidazolinone herbicides is usually due to enhanced metabolism or substitution of an amino acid in the ALS protein sequence in the tolerant plant species relative to the susceptible species, although differential uptake can contribute to this tolerance (Brown and Neighbors 1987; Eberlein et al. 1999; Wilcut et al. 1989). Reddy and Bendixen (1988) attributed the overall susceptibility of purple and yellow nutsedge to chlorimuron to be due to its slow rate of degradation. Basipetal movement of imazaquin to meristems of basal bulbs, roots, and rhizomes of purple and yellow nutsedge has been reported (Nandihalli and Bendixen 1988). Vencill (1998) observed that foliar-applied ^{14}C -pyriithiobac was mostly translocated to the shoots of yellow nutsedge plants. Because of the potential of CGA-362622 to control purple and yellow nutsedge, its physiological behavior in these species should be investigated. Therefore, objectives of this research were to evaluate the absorption, translocation, and metabolism of foliar-applied ^{14}C -CGA-362622 in purple and yellow nutsedge.

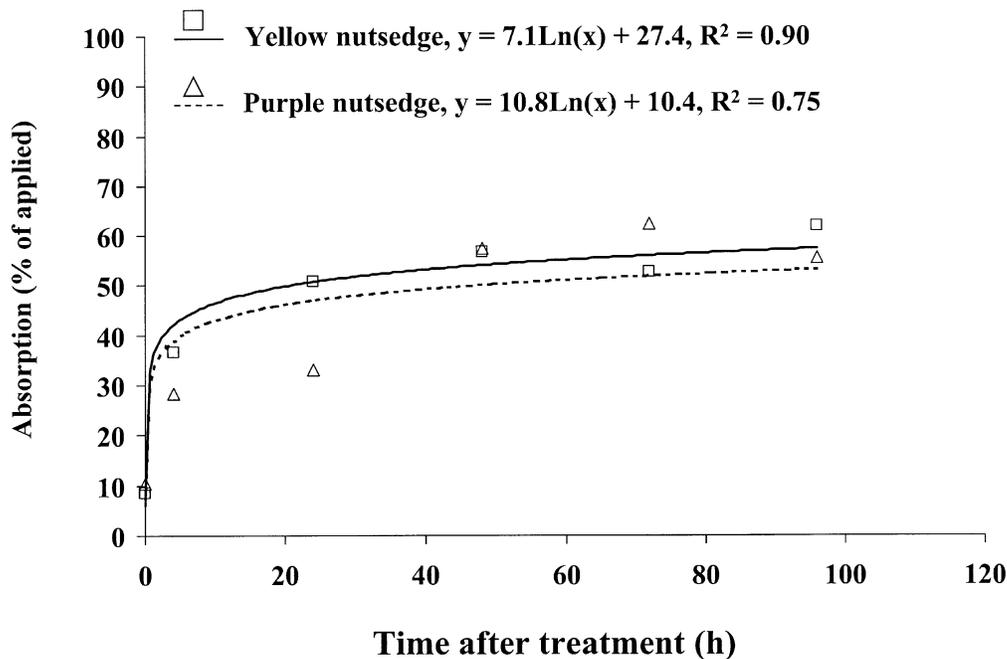


FIGURE 1. Percent absorption of ^{14}C -CGA-362622 by purple and yellow nutsedge (*Cyperus rotundus* and *C. esculentus*) over time.

Materials and Methods

Plant Material

Tubers of purple and yellow nutsedge were planted in a 1:1 mixture of pure sand and Norfolk loamy sand (fine-loamy, siliceous, thermic, Typic Paleudults) in 10- by 10-cm square plastic pots. This soil is typical for cotton production on the Mid-Atlantic Coastal Plain and Southeastern Coastal Plain. The nutsedge species were thinned to 1 plant pot^{-1} upon emergence. Plants were watered daily and maintained in a glasshouse at approximately 24 ± 2 C in a 16 h photoperiod of natural and supplemental metal halide lighting with an average midday photosynthetic photon flux of $700 \mu\text{mol m}^{-2} \text{s}^{-1}$. When plants reached the six-leaf stage, they were moved into a growth chamber with 26 C constant temperature and approximately 50% relative humidity. Growth chamber lighting was provided by fluorescent and incandescent lamps at $500 \mu\text{mol m}^{-2} \text{s}^{-1}$. Plants were allowed to acclimate for 3 d before treatment with ^{14}C -CGA-362622. A 1-cm^2 area on the middle-axial surface of the third fully expanded leaf was marked on purple and yellow nutsedge and covered with a plastic bag before overspraying. CGA-362622 was applied to both nutsedge species at 6 g ai ha^{-1} using a moving belt spray chamber calibrated to supply 190 L ha^{-1} at 146 kPa immediately before spotting with ^{14}C -CGA-362622. This rate of CGA-362622 is within the rate range proposed for registration (Holloway et al. 2000; Hudetz et al. 2000; Wells et al. 2000). Two studies were conducted as randomized complete blocks with split-split-plot treatment designs and three replications of treatments to evaluate absorption, translocation, and metabolism of CGA-362622. The five harvest timings were considered as main plots, two nutsedge species as subplots, and division of plant parts as subsubplots. Each study was repeated once.

Absorption and Translocation

Technical grade [pyridinyl-2- ^{14}C]-CGA-362622 (Figure 1) with $450.6 \text{ kBq } \mu\text{mol}^{-1}$ specific activity and 97.8% radiochemical purity was used for the study. Five $1\text{-}\mu\text{l}$ droplets¹ of ^{14}C -CGA-362622 dissolved in high-performance liquid chromatography-grade water with 0.25% nonionic surfactant² and containing approximately 2.9 kBq of radioactivity were placed within the marked area on the third fully expanded leaf of purple and yellow nutsedge.

Plants were removed from soil 4, 24, 48, 72, or 96 h after treatment and divided into treated leaf area, aerial portions above and below the treated leaf area, and other leaves, roots, and tubers. In addition, three replications of each species were spotted with ^{14}C -CGA-362622 and harvested after 10 s to evaluate the efficiency of the leaf wash technique. The treated leaves were rinsed slowly with 10 ml of methanol-water (1:1, v/v) and 0.25% (v/v) nonionic surfactant² to remove nonabsorbed ^{14}C -CGA-362622. This leaf wash technique has greater than 91% efficiency and ^{14}C recovery from parafilm did not change over time (data not shown), thereby indicating that nonbiological losses were negligible (Askew and Wilcut 2002). A 1-ml aliquot of the rinse was added to 25 ml of the scintillation fluid,³ and radioactivity was quantified by liquid scintillation spectrometry (LSS).⁴ All plant parts, including roots, were dried for 48 h at 40 C, weighed, and combusted with a biological sample oxidizer.⁵ Radioactivity in the oxidized samples was quantified by LSS.

Data were subjected to analysis of variance (ANOVA) with sums of squares partitioned to reflect a split-split-plot treatment structure and trial effects. The five harvest timings were considered main plots, the two nutsedge species were considered subplots, and the seven portions of quantified radioactivity (wash, treated leaf, foliage above treated leaf, foliage below treated leaf, other leaves, tubers, and roots)

were considered subplots. Log transformation improved homogeneity of variance on the basis of inspection of plotted residuals; therefore, data were transformed before ANOVA. Trial effects were considered random, and mean squares were tested on the basis of the treatment design (McIntosh 1983). Where main-plot effects were significant, regressions were used to explain the relationship of measured responses over time. On the basis of ANOVA, significant main effects were averaged over harvest times (main plots) and separated by Fisher's Protected LSD test at $P = 0.05$. Because data transformation did not alter mean rank, mean separation of main effects was performed on nontransformed data for clarity.

Metabolism

Plants were grown and treated as in the translocation study; however, they were treated with 5.1 kBq of ^{14}C -CGA-362622. At plant harvest, partitioned plant parts were immediately stored at -30 C until further analysis. Only the treated leaf area and other leaves were used for the analysis of metabolites.

Plant parts were ground in a tissue homogenizer⁶ with 10 ml of acetonitrile-water (4:1, v/v). The homogenate was rinsed through a vacuum filtration apparatus with an additional 10 to 15 ml of solvent. The washed residue and filter paper⁷ from the homogenizer were dried at room temperature, weighed, and oxidized, and radioactivity was determined by LSS. The filtrate was evaporated to dryness at 32 C under a stream of air. The samples were then brought to a 1-ml volume with acetonitrile, shaken for 30 min on a rotary shaker,⁸ and stored at 4 C until further analysis. To evaluate the potential effects of the extraction process on herbicide degradation, fresh plant leaves were harvested, spotted with five 1- μl droplets of the ^{14}C herbicide solution, and immediately processed in conjunction with the study samples. All herbicide extraction techniques were conducted on these freshly spotted leaves so that effects of extraction could be determined by later comparing the pure ^{14}C -CGA-362622 standard with the fresh leaf extraction.

Fifty microliters from each extraction sample was spotted on 20- by 20-cm silica gel thin layer chromatography (TLC) plates⁹ and developed to 16 cm. The solvent consisted of chloroform-methanol-ammonium hydroxide-water (80:30:4:2, v/v) (Askew and Wilcut 2002), according to the Syngenta recommendation. Plates were partitioned with a ruler into ten 1.8-cm-wide lanes. A standard that consisted of 5 μl of ^{14}C -CGA-362622 herbicide solution dissolved in 1 ml acetonitrile was spotted on the first lane of each plate. The remaining nine lanes received a single replicate of the treated leaf or other leaves sample from each of the two species for the two runs of the study.

Plates were air-dried, and radioactive positions, quantities, and corresponding R_f values were determined by scanning TLC plates with a radiochromatogram scanner.¹⁰ Radioactive peaks were integrated using the Win-Scan[®] software,¹¹ with smoothing set to 13-point cubic and background excluded from peak area calculation. Peaks below 1% of the total radioactivity were rejected. The parent herbicide was identified by comparing the R_f value from the corresponding standard. Data consisted of the percentage parent herbicide, the sum percentage of all metabolites that were more polar than the parent herbicide, and the sum percentage of all

TABLE 1. Percentage of applied radioactivity recovered from nutsedge species (*Cyperus* spp.) after foliar treatment of ^{14}C -CGA-362622, averaged over trials and harvest at 4, 24, 48, 72, and 96 h after treatment.^{a,b}

Description of recovery area	Purple nutsedge	Yellow nutsedge
	%	
Nonabsorbed	52	48
Treated leaf area	6	6
Above treated leaf area	5	5
Below treated leaf area	11	13
Other leaves	11	14
Tubers	2	< 1
Roots	< 1	3
LSD (0.05)	5	3
Recovery	88	90

^a The third fully expanded leaves of both species were treated with five 1- μl droplets of ^{14}C -CGA-362622 dissolved in water and 0.25% (v/v) nonionic surfactant and containing approximately 2.9 kBq radioactivity.

^b Purple nutsedge, *Cyperus rotundus*; yellow nutsedge, *Cyperus esculentus*.

metabolites that were less polar than the parent herbicide. Statistical procedures were similar to the uptake and translocation study.

Results and Discussion

Absorption and Translocation

According to ANOVA, trial effects were not significant for any harvest timing for ^{14}C -CGA-362622 absorption; thus, data were pooled over trials. Absorption, based on leaf wash recovery subtracted from 100 and total ^{14}C recovered from plant parts, followed logarithmic trends for both purple and yellow nutsedge (Figure 1). Most of the herbicide was absorbed before 24 h in both species. But only about 55% of the herbicide was absorbed by 96 h after treatment of purple and yellow nutsedge. Similar results were observed in other species sensitive to CGA-362622 (Askew and Wilcut 2002). By comparison, purple and yellow nutsedge absorbed ^{14}C -imazaquin 53 and 57%, respectively, 8 d after treatment (Nandihalli and Bendixen 1988), whereas less than 35% of ^{14}C -chlorimuron was absorbed 8 d after treatment in both species (Reddy and Bendixen 1988). Yellow nutsedge absorbed 50% of foliar-applied ^{14}C -pyrithiobac (Vencill 1998). Dayan et al. (1996) reported that the relatively high amount of wax present on the surface of nutsedge leaves may be a factor limiting sulfentrazone foliar absorption relative to other weeds lacking similar epicuticular wax.

Trial effects were not significant for harvest timing for the translocation of ^{14}C ; thus, the amounts of radioactivity in both purple and yellow nutsedge, as percentages of applied ^{14}C , were averaged over trials and harvest timings (Table 1). Only 6% of applied ^{14}C remained in the treated leaf area, whereas more than 30% moved out of the treated leaf area in both nutsedge species. By comparison, greater than 70% of absorbed ^{14}C -chlorimuron and ^{14}C -imazaquin remained in the treated area of purple and yellow nutsedge 192 h after treatment (Nandihalli and Bendixen 1988; Reddy and Bendixen 1988). Askew and Wilcut (2002) observed that less than 2% of applied ^{14}C -CGA-362622 moved out of the treated leaves of cotton, peanut (*Arachis hypogaea* L.), and sicklepod [*Senna obtusifolia* (L.) Irwin and Barneby].

Wilcut et al. (1989) showed that translocation of ^{14}C -chlorimuron out of the treated leaf in several plant species was less than 20% of the applied radioactivity.

Translocation of radioactivity was both acropetal and basipetal in both nutsedge species. Five percent of applied ^{14}C moved acropetally in both purple and yellow nutsedge (Table 1). Typically, sulfonyleurea herbicides are more readily translocated in the xylem than in the phloem (Hageman and Behrens 1984; Lycan and Hart 1999; Petersen and Swisher 1985). However, greater than 24% of ^{14}C -CGA-362622 was translocated basipetally in both species. Past research has observed limited basipetal movement of sulfonyleurea herbicides in nutsedge species (Nandihalli and Bendixen 1988; Reddy and Bendixen 1988). Significant differences among ^{14}C accumulation values in the different plant parts occurred in both nutsedge species (Table 1). Greater than 22% of applied ^{14}C accumulated below the treated leaf area and other leaves in both nutsedge species, whereas less than 4% accumulated in tubers and roots. Past studies have observed similar results concerning the translocation of sulfonyleurea herbicides in nutsedge species (Nandihalli and Bendixen 1988; Reddy and Bendixen 1988). Greater herbicide movement to meristematic regions may contribute to greater herbicidal activity because ALS is most active in developing tissue (Gerwick et al. 1993). Low accumulation of ^{14}C in tubers and roots in the current study may have been due to the inactivity of metabolic sinks at this period of plant growth (Akobundu et al. 1969; Reddy and Bendixen 1988). Translocation of ^{14}C in both nutsedge species was similar.

Metabolism

The plant extraction process was very effective, with less than 1% of the absorbed ^{14}C remaining in any plant residue (data not shown). The three most common metabolites of ^{14}C -CGA-362622 formed in purple and yellow nutsedge occurred at $R_f = 0.15$, 0.25, and 1.03 (Figure 2). The R_f value of CGA-362622 was 0.5 (Askew and Wilcut 2002). Any peak identified by the software that had an R_f value less than 0.5 was considered more polar than CGA-362622, and R_f values greater than 0.5 were combined as less polar metabolites (Askew and Wilcut 2002). Harvest time, plant part, and species effects were not significant for percentages of polar and nonpolar metabolites produced ($P > 0.05$). The effects at the time of harvest were significant for percentage of parent ^{14}C -CGA-362622 in purple and yellow nutsedge; however, percentage of the parent herbicide in the treated leaf and other leaves was statistically equivalent (Figure 3). Both nutsedge species metabolized CGA-362622 within 4 h of treatment. Plant extraction techniques resulted in greater than 90% ^{14}C recovery based on the extraction of freshly spotted samples (data not shown). Nutsedge species and metabolite compound polarity averaged over harvest intervals are presented in Table 2. Only 28 and 33% of the ^{14}C -CGA-362622 parent herbicide remained in purple and yellow nutsedge, respectively, when averaged over harvest intervals and plant parts. But the amount of CGA-362622 remained fairly constant after 24 h (Figure 3). By comparison, chlorimuron degradation did not exceed 15% of the absorbed parent herbicide in yellow nutsedge or 26% of it in purple nutsedge 192 h after treatment (Reddy and Bendixen 1988). Askew and Wilcut (2002) reported that

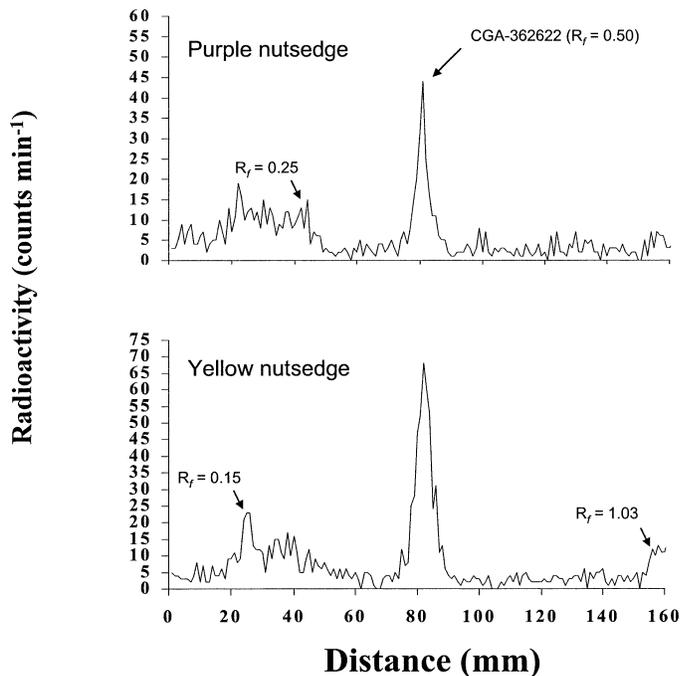


FIGURE 2. Radioactivity traces from thin layer chromatographic separations of ^{14}C -CGA-362622 and its metabolites formed by leaves of purple and yellow nutsedge (*Cyperus rotundus* and *C. esculentus*) after 72 h.

between 59 and 63% of the parent CGA-362622 was not metabolized in sensitive species, whereas that between 31 and 43% was not metabolized in tolerant species. Less than 10% of nicosulfuron was metabolized within 72 h in sensitive broadleaf signalgrass [*Brachiaria platphylla* (Griseb.) Nash] (Gallaher et al. 1999). Extrapolated half-life of CGA-362622, as calculated by other researchers for this and other herbicides (Askew and Wilcut 2002; Newsom et al. 1993; Shaner and Robson 1985; Wilcut et al. 1989), is no more than 4 h in both purple and yellow nutsedge.

Polar metabolites relative to CGA-362622 were produced in the greatest percentages (65 and 58%) in purple and yellow nutsedge, respectively, averaged over harvest intervals and plant parts. Only 7% of the parent compound was metabolized into nonpolar metabolites in both species. Polar metabolites were produced in greater percentages than nonpolar metabolites in both sensitive and tolerant species (Askew and Wilcut 2002), suggesting that the proportion of polar to nonpolar metabolites does not affect sensitivity. The metabolites at the R_f values of 0.15 and 0.25 were the largest contributors to total polar metabolites, whereas the metabolite at $R_f = 1.00$ was the largest contributor to total nonpolar metabolites. Similar metabolites at R_f values of 0.15 and 1.00 were found at corresponding R_f values in cotton, jimsonweed (*Datura stramonium* L.), peanut, and sicklepod (Askew and Wilcut 2002). However, purple and yellow nutsedge produced an extra metabolite at $R_f = 0.25$ (Figure 2), which was not identified in cotton, a species tolerant to CGA-362622 (Askew and Wilcut 2002). This extra metabolite may exhibit some degree of herbicidal activity that could elucidate the reasons for the susceptibility of purple and yellow nutsedge to CGA-362622. Hodges et al. (1990) observed that the metabolites of an unspecified triazolopyrimidine herbicide (another herbicide family that inhibits ALS) still retained herbicidal properties.

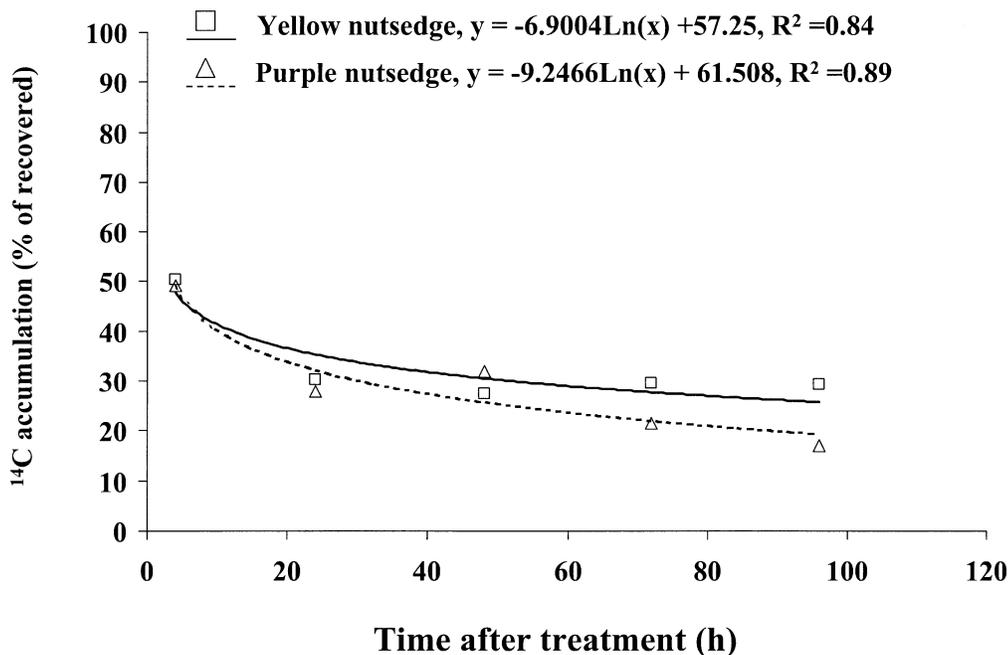


FIGURE 3. Decline of ^{14}C -CGA-362622 concentration in purple and yellow nutsedge (*Cyperus rotundus* and *C. esculentus*) over time.

Susceptibility of purple and yellow nutsedge to chlormuron may be attributed to its slow rate of degradation (Reddy and Bendixen 1988). Numerous studies have reported differential metabolism as conferring differential tolerance to sulfonylurea herbicides (Askew and Wilcut 2002; Green and Ulrich 1993; Moreland et al. 1996; Neighbors and Privalle 1990; Wilcut et al. 1989). The rapid absorption and high degree of translocation when compared with other sulfonylurea and imidazolinone herbicides, as well as the production of a potentially herbicidally active metabolite, may explain the susceptibility of purple and yellow nutsedge to CGA-362622. Basipetal translocation of CGA-362622 placed the herbicide in the meristematic regions of foliar vegetative growth. Reduction in leaf area of nutsedge plants can limit the amount of carbohydrates necessary for tuberization (Akin and Shaw 2001). The small percentage of parent CGA-362622 in both nutsedge species demonstrates the

TABLE 2. Percentage of radioactivity traces from thin layer chromatographic separations of CGA-362622 and its metabolites from treated and other leaves of nutsedge species (*Cyperus* spp.). Data are averaged over trials and harvest at 4, 24, 48, 72, and 96 h after treatment.^{a,b}

Description of compounds	Purple nutsedge	Yellow nutsedge
	%	
Polar metabolites	65	58
CGA-362622, parent	28	33
Nonpolar metabolites	7	7
LSD (0.05)	15	13

^a The third fully expanded leaves of both species were treated with five 1- μl droplets of ^{14}C -CGA-362622 dissolved in water and 0.25% (v/v) nonionic surfactant and containing approximately 5.1 kBq radioactivity. Polar metabolites indicates the percentage sum of all metabolites more polar than CGA-362622, and "nonpolar metabolites" indicates the percentage sum of all metabolites less polar than CGA-362622.

^b Purple nutsedge, *Cyperus rotundus*; yellow nutsedge, *Cyperus esculentus*.

apparent level of the highly toxic activity of this herbicide. However, soil activity of CGA-362622 contributes to the susceptibility of purple and yellow nutsedge to postemergence herbicides (J. S. McElroy, unpublished data). These factors illustrate the potential of CGA-362622 for effective control of both purple and yellow nutsedge that has been documented in greenhouse and field management experiments (Porterfield et al. 2002a; Troxler et al. 2002). Additional research should investigate the physiological behavior of root-absorbed CGA-362622 in both nutsedge species and the effect of ALS enzyme inhibition by CGA-362622 in susceptible (nutsedge) and tolerant (cotton) species.

Sources of Materials

¹ Hamilton® Microliter Pipetter No. 705, Hamilton Co., 4970 Energy Way, Reno, NV 89502.

² Induce® nonionic low foam wetter-spreaders adjuvant contains 90% nonionic surfactant (alkylaryl polyoxyalkane ether and isopropanol), free fatty acids, and 10% water, Helena Chemical Company, Suite 500, 6075 Poplar Avenue, Memphis, TN 38137.

³ ScintiVerse® SX18-4 Universal Liquid Scintillation Cocktail, Fisher Scientific, 1 Reagent Road, Fair Lawn, NJ 07410.

⁴ Packard TRI-CARB 2100TR Liquid Scintillation Spectrometer, Packard Instrument Company, 2200 Warrenville Road, Downers Grove, IL 60515.

⁵ Model OX-500 Biological Material Oxidizer, R. J. Harvey Instrument Corp., 123 Patterson Street, Hillsdale, NJ 07642.

⁶ Pyrex® Tissue Homogenizer No. 7727-40, Corning Inc., 1 Riverfront Plaza, Corning, NY 14831.

⁷ Whatman® Filter Paper 55mm, Whatman International Ltd., Whatman Holst Leonards Road Allington Maidstone, Kent ME160LS, U.K.

⁸ Lab-Line Orbit Environ-Shaker, Lab-Line Instruments, Inc., 1999 North 15th Avenue, Melrose Park, IL 60160.

⁹ Whatman K6F Silica Gel 60A thin layer chromatography plates, Whatman Inc., 9 Bridewell Place, Clifton, NJ 07013.

¹⁰ BioScan System 200 Imaging Scanner, Bioscan, 4590 MacArthur Boulevard NW, Washington, DC 20007.

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