Chapter VI

Absorption, Translocation, and Metabolism of CGA 362622 in Cotton, Spurred Anoda (*Anoda cristata*), and Smooth Pigweed (*Amaranthus hybridus*)

Abstract: Absorption, translocation, and metabolism of CGA 362622 were studied in cotton, spurred anoda, and smooth pigweed. Cotton and weed seedlings were treated with foliar applied ^{14}C-labeled CGA 362622 at the following growth stages: cotton at the cotyledon to one-leaf stage; cotton at the two- to three-leaf growth stage; spurred anoda and smooth pigweed at the four- to six-leaf growth stage. Treated seedlings were harvested at 6, 24, 48, and 72 hours after treatment (HAT). Absorption of ^{14}C-CGA 362622 was lower in cotton at the two- to three-leaf growth stage than in cotton at the cotyledon to one-leaf growth stage or in the weed species. Two- to three-leaf cotton absorbed 39% of ^{14}C-CGA 362622 at 42 HAT, while cotyledon to one-leaf cotton, spurred anoda, and smooth pigweed absorbed 55 to 59% of CGA 362622. Most of the radioactivity absorbed by smooth pigweed was translocated to the stem, leaves above the treated leaf and leaves below the treated leaf, but not to the root. Translocation of absorbed ^{14}C out of the treated leaf was minimal in spurred anoda and in cotton at both growth stages. Metabolism of ^{14}C CGA 362622 was more rapid in cotton than in spurred anoda or smooth pigweed. At 6 HAT, 30 to 31% of the absorbed ^{14}C had been metabolized in cotton compared to 15 to 18% metabolized in spurred anoda and smooth pigweed. By 72 HAT, metabolism of ^{14}C was greatest in cotton at the cotyledon to one-leaf stage of growth. According to results, differential absorption, translocation, and metabolism contribute to the differential tolerance of cotton, spurred anoda, and smooth pigweed to the herbicide CGA 362622. Rapid translocation and slow rate of metabolism appear to explain the susceptibility of smooth pigweed to this herbicide. Reduced absorption and translocation and rapid metabolism contribute to the CGA 362622 tolerance of cotton at both growth stages. Limited translocation may explain the intermediate tolerance of spurred anoda to the herbicide CGA 362622.

Nomenclature: CGA 362622, *N*-[(4,6-dimethoxy-2-pyrimidinyl)carbamoyl]-3-(2,2,2-trifluoroethoxy)-pyridin-2-sulfonamide sodium salt; cotton, *Gossypium hirsutum* L.; smooth

pigweed, *Amaranthus hybridus* L. #[1] AMACH; spurred anoda, *Anoda cristata* (L.) Schlecht. # ANVCR.

Additional index words: Sulfonylurea herbicides, herbicide tolerance, herbicide sensitivity.

Abbreviations: HAT, hours after treatment.

INTRODUCTION

CGA 362622 [N-({4,6-dimethoxy-2-pyrimidinyl}carbamoyl)-3-(2,2,2-trifluoroethoxy)-pyridin-2-sulfonamide sodium salt] is an experimental sulfonylurea herbicide being developed by Syngenta Crop Protection, Inc. Sulfonylurea herbicides inhibit the acetolactate synthase enzyme (ALS, EC 4.1.3.18), which serves as the first common enzyme in the formation of the branched-chain amino acids valine, leucine, and isoleucine (Shimzu et al. 1994). Crop tolerance to ALS inhibitors is generally due to rapid metabolism of the parent molecule into non-herbicidal metabolites (Brown and Neighbors 1987; Wilcut et al 1989; Walker et al. 1994; Hinz and Owen 1996).

CGA 362622 has low mammalian toxicity, a favorable environmental profile, low use rates, and a broad spectrum of weed control (Hudetz et al. 2000). It is being evaluated for weed control in cotton (*Gossypium hirsutum* L.), sugarcane (*Saccharum officinarum* L.), and several minor crops (Hudetz et al. 2000). Postemergence (POST) applications of CGA 362622 generally result in transient cotton injury. Results of studies conducted in Louisiana demonstrated no visible cotton response to CGA 362622 (Vidrine and Miller 2001). However, reports of early crop injury are more common. Symptoms of chlorosis or stunting with rapid crop recovery and no effect on yield have been reported in multiple locations (Brecke et al. 2000; Faircloth et al. 2001). In North Carolina, injury up to 40% has been observed, although symptoms were also transient (Wilcut et al. 2000).

[1] Letters following this symbol are a WSSA-approved computer code from *Composite List of Weeds*, Revised 1989. Available from WSSA, 810 East 10th Street, Lawrence, KS 66044-8897.

Application of CGA 362622 POST controls many broadleaf weeds including common lambsquarters (*Chenopodium album* L.), entireleaf morningglory [*Ipomoea hederacea* (L.) Jacq.], palmer amaranth (*Amaranthus palmeri* S. Wats.), pitted morningglory (*I. lacunosa* L.), sicklepod (*Cassia obtusifolia* L.), slender amaranth (*Amaranthus gracilis* Desf.), smooth pigweed (*Amaranthus hybridus* L.), and tall morningglory [*Ipomoea purpurea* (L.) Roth] (Wilcut et al. 2000; Porterfield et al. 2000). Suppression of other troublesome weeds such as purple nutsedge (*Cyperus rotundus* L.) and johnsongrass [*Sorghum halepense* (L.) Pers.] can also be achieved (Hudetz et al. 2000). However, CGA 362622 will not control smallflower morningglory [*Jacquemontia tamnifolia* (L.) Griseb.], prickly sida (*Sida spinosa* L.), or spurred anoda [*Anoda cristata* (L.) Schlecht.] (Brecke et al. 2000; Faircloth et al. 2001). In field studies at the Eastern Shore Agricultural Research and Extension Center, control of solanaceous weeds from CGA 362622 application was low.

The structure of CGA 362622 closely resembles that of two other sulfonylureas, nicosulfuron and rimsulfuron (Figure 6.1). Corn tolerance to nicosulfuron and rimsulfuron is primarily due to rapid metabolism of the parent molecule into non-herbicidal metabolites (Gallaher et al 1999; Hinz and Owen 1996; Carey et al. 1997; Koeppe et al. 2000). Low absorption and translocation rates may also contribute to nicosulfuron tolerance in corn (Hinz and Owen 1996). However, tolerance of certain weeds to these herbicides may not involve metabolism. Ackley et al. (1999) reported that differential tolerance of three nightshade species to rimsulfuron was not through metabolism, but might have been due to different levels of absorption or translocation. Also, eastern black nightshade tolerance to nicosulfuron has been attributed to lower sensitivity of the ALS enzyme and to low translocation of the herbicide (Carey et al. 1997). Likewise, the mechanism of resistance for many ALS-resistant weeds is due to reduced enzyme affinity for ALS inhibitors (Manley 1996; Lovell et al. 1996; Sprague et al. 1997;bFoes et al. 1998).

Askew and Wilcut (2002) evaluated absorption, translocation, and metabolism of ^{14}C-CGA 362622 in cotton, peanut (*Arachis hypogaea* L.), jimsonweed (*Datura stramonium* L.), and sicklepod. They reported metabolism as the basis of selectivity between the tolerant plants (cotton and jimsonweed) and the susceptible plants (peanut and sicklepod). Significant

translocation of radiolabeled CGA 362622 was observed only in jimsonweed, and CGA 362622 absorption was lower in cotton compared to other species (Askew and Wilcut 2002).

Visual observations by the authors indicate that cotton is more susceptible to CGA 362622 applications at the cotyledon stage of growth. Reasons for differential response of cotton at different growth stages to CGA 362622 have not been investigated. Similarly, the physiological basis for spurred anoda tolerance and smooth pigweed susceptibility to CGA 362622 application has not been reported. The objective of this research was to determine the physiological basis for the observed differential response of cotton at different stages of growth, and the differential response of spurred anoda and smooth pigweed to CGA 362622. Therefore, studies were conducted to evaluate absorption, translocation, and metabolism of foliar-applied ^{14}C-labeled CGA 362622 in these species.

MATERIALS AND METHODS

Chemicals. Formulated and radiolabeled samples of CGA 362622 were provided by Syngenta Crop Protection, Inc. The specific activity of [pyridinyl-2-^{14}C]-CGA 362622 was 1898 kBq/mg and radiochemical purity was 97.6%.

Plant material. Seeds of spurred anoda and smooth pigweed were collected from plants at the Eastern Shore Agricultural Research and Extension Center near Painter, VA. Seeds were planted into 43 by 53-cm greenhouse flats[2] containing a commercial potting mix[3]. Weed seedlings were allowed to develop until emergence of a true leaf before being transplanted into 9.5 by 9.5-cm[4]

[2] Sutton universal greenhouse flat. Inside dimensions 51 cm by 40 cm by 5.7 cm. Wetzel, Inc., 1345 Diamond Springs Road, Virginia Beach, VA 23455.

[3] Pro-Mix BX. Premier Horticulture, Inc., Red Hill, PA 18076.

[4] T. O. Plastics 4" Fill Pots. Inside dimensions 9.5 by 9.5 by 8.1 cm. Wetzel, Inc., 1345 Diamond Springs Road, Virginia Beach, VA 23455.

pots filled with a 1:1 mixture of potting soil and vermiculite[5]. Cotton variety 'Sure Grow 125' was planted at four seeds per pot in 9.5 by 9.5-cm pots containing the same potting mixture and thinned to two cotton plants per pot after emergence. Plants were watered as needed and maintained in a glasshouse under natural and supplemental metal halide lighting (650 µmol/m^2/s photon flux) with a photoperiod of 14-h. All plants were transferred to glass bottles containing one-quarter strength Hoagland's solution (pH 6.5) 24 hours before treatment.

Foliar absorption and translocation. Prior to treatment with radiolabeled CGA 362622, plants were treated with 3.8 g ai/ha of formulated CGA 362622 with 0.25% v/v non-ionic surfactant (NIS)[6]. CGA 362622 was applied using a moving nozzle, compressed air, greenhouse sprayer equipped with a flat fan spray tip[7] delivering 170 L/ha at 290 kPa. Immediately following application of formulated CGA 362622, 10 µL of ^{14}C-CGA 362622 containing 3.4 kBq of radiolabeled CGA 362622 was dissolved in aqueous ethanol and a nonionic surfactant (0.5% v/v). Seedlings of spurred anoda and smooth pigweed were treated at the four- to six-leaf stage of growth. Drops with the labeled herbicide were applied to the top leaf surface of all plants and to the third-youngest leaf of spurred anoda and smooth pigweed. In cotton, drops were applied to a cotyledon leaf of cotton seedlings at the cotyledon to one-leaf growth stage, and to the second oldest true leaf of cotton seedlings at the two- to three-leaf growth stage. All plants were maintained in a greenhouse until harvest.

Plants were removed from Hoagland's solution at 6, 24, 48, and 72 HAT. Roots were blotted dry and plants were divided into treated leaf, foliage below treated leaf, foliage above treated leaf, roots, and stem. Treated leaves were rinsed in 20 mL of water:methanol (9:1) for 0.5 min to

[5] Vermiculite Medium Grade #3. Wetzel, Inc., 1345 Diamond Springs Road, Virginia Beach, VA 23455.

[6] Induce non-ionic low foam wetter/spreader adjuvant with 90% principal functioning agents as a blend of alkyl aryl polyoxylkane ether free fatty acids. Helena Chemical Company, 5100 Poplar Avenue, Memphis, TN 38137.

[7] Teejet 8001 E flat fan spray tip. Spraying Systems Company, North Avenue, Wheaton, IL 60188.

remove unabsorbed radioactivity. A 1 mL aliquot of the leaf rinse was added to 15 mL of scintillation cocktail[8] and radioactivity was quantified by liquid scintillation spectrometry (LSS)[9]. Plant sections were combusted with a biological sample oxidizer[10], and radioactivity was trapped as $^{14}CO_2$ and quantified by LSS. Absorption was expressed as a percent of total applied ^{14}C, based on radioactivity recovered in the leaf wash and in the combusted plant sections. Translocation was expressed as a percentage of the absorbed radioactivity.

Autoradiography. Plants were grown and treated as described in the absorption and translocation studies. Plants were removed from Hoagland's solution at 6, 24, 48, and 72 HAT with the radiolabeled herbicide. Roots were blotted dry and plants were glued to paper and pressed until dry. Dried plants were exposed to X-ray film[11] for 21-d, and the film was then developed to visually express any movement of ^{14}C-CGA 362622 or metabolites.

CGA 362622 metabolism. Plants were grown and treated as described in the absorption and translocation studies. Parent herbicide and possible metabolites were extracted from plant tissues according to the methods of Askew and Wilcut (2002) with minor modifications. Cotton and weed seedlings were removed from Hoagland's solution at 6, 24, 48, and 72 HAT. Since the major portion of absorbed radioactivity remained at the treated leaf, metabolism studies were conducted only with treated leaves. After harvest, treated leaves were frozen in liquid nitrogen, pulverized with mortar and pestle, and homogenized in 5 mL of acetonitrile:water (4:1, v/v). Homogenates were centrifuged at $2{,}000 \times g$ for 5 min, and the supernatant was saved. The solid portion was extracted two more times with 5 mL of 80% acetonitrile and centrifuged. The supernatants were concentrated to 1 mL by evaporation. Standard ^{14}C-labeled CGA 362622 (3

[8] Scintiverse. Fisher Scientific, 50 Fadem Road, Springfield, NJ 07081-3193.

[9] Liquid Scintillation Spectrometer, Beckman Model LS-5000TA. Beckman Instrument Co., Fullerton CA 92634.

[10] Packard Sample Oxidizer Model 307. Packard Instrument Co., Downers Grove, IL 60515.

[11] Kodac X-OMAT AR film. Eastman Kodak Co., Rochester, NY 14650.

μL) and 50 μL of each sample were loaded on silica gel plates[12] for thin layer chromatography (TLC) analysis. Prior to sample loading, TLC plates were activated by heating at 65 C for 4 h. Plates were run in duplicate and developed in a chloroform:methanol:ammonium hydroxide:water (80:30:4:2, v/v/v/v) solvent system.

Developed TLC plates were exposed to X-ray film[11] for 21-d, and the film was then developed to reveal the positions of radioactive bands on the plates. Radioactive bands were then scraped from the plate and the amount of radioactivity was determined by LSS. Metabolites were separated by their ratio of front (Rf) values, and the radioactivity detected in each metabolite is expressed as a percentage of the radioactivity recovered in the TLC analysis.

Statistical analyses. Absorption, translocation, and metabolism studies were completely randomized designs with two replications of each treatment. Absorption and translocation studies were repeated three times and metabolism studies were repeated twice. Analysis of variance was performed, and data from separate studies were combined and averaged over experiments. Treatment means were separated using Fisher's protected LSD test at the 0.05 significance level.

RESULTS AND DISCUSSION

Foliar absorption and translocation. Absorption of CGA 362622 into spurred anoda and smooth pigweed was rapid (Table 6.1). At 6 HAT, 42 and 55% of applied ^{14}C were absorbed into spurred anoda and smooth pigweed, respectively. The amount of ^{14}C absorbed by spurred anoda and smooth pigweed did not increase appreciably with increases in exposure time. Absorption of ^{14}C into cotton seedlings was slower, with only 24% absorbed after 6-h at both growth stages of cotton. Absorption of ^{14}C increased with time only in cotton seedlings at the cotyledon to one-leaf stage. At 48 HAT, absorption peaked with 59% in cotyledon to one-leaf cotton, 39% in two- to three-leaf cotton, 55% in spurred anoda, and 57% in smooth pigweed.

[12] Silica Gel 60 F_{254} precoated TLC plates. EM Science, 480 Democrat Road, Gibbstown, NJ 08027.

Translocation of absorbed ^{14}C out of the treated leaf was appreciable only in smooth pigweed (Table 6.1). Greater than 92% of the absorbed ^{14}C remained in the treated leaf of cotton seedlings at both growth stages. Translocation out of the treated leaf of cotton seedlings ranged from 5% to 7 % of absorbed ^{14}C. More than 80% of absorbed ^{14}C remained in the treated leaf of spurred anoda. Translocation to other plant parts of spurred anoda was less than 8%. In contrast, at least 42% of absorbed ^{14}C translocated out of the treated leaf of smooth pigweed. By 6 HAT, 37% of absorbed ^{14}C had moved into the stem of smooth pigweed. Translocation to foliage above the treated leaf was between 17 and 31% of absorbed ^{14}C in smooth pigweed at any time after treatment. Accumulation of absorbed ^{14}C in foliage below the treated leaf was 1 to 22% in smooth pigweed. Translocation to roots was minimal in all plant species.

Autoradiography of treated seedlings supports the results of the absorption and translocation studies. At 72 HAT, movement of ^{14}C out of the treated leaf was minimal in cotton at both growth stages (Figure 6.2A and 6.2B). Translocation of ^{14}C in spurred anoda was also limited (Figure 6.2C). However, significant translocation of ^{14}C out of the treated leaf was evident in smooth pigweed (Figure 6.2D).

Results from absorption and translocation studies are generally similar to recent reports of other investigators. Cotton and peanut absorbed 26% of CGA 362622, while absorption in sicklepod and jimsonweed was 32% and 53%, respectively (Askew and Wilcut 2002). The same researchers also reported that translocation of applied ^{14}C out of the treated leaf of cotton, peanut, and sicklepod was less than 3%. Translocation was greater in jimsonweed with 23 to 35% of absorbed ^{14}C moving to foliage above the treated leaf (Askew and Wilcut 2002). Reported translocation of other ALS-inhibiting herbicides such as chloransulam methyl, chlorimuron, and nicosulfuron in smooth pigweed were similar to the results of the present study. None of the three herbicides moved to the root of smooth pigweed, and translocation of nicosulfuron and chlorimuron did not increase appreciably with increases in exposure time (Manley et al. 1999; Poston et al. 2002). Likewise, the tolerance of spurred anoda to thifensulfuron and of eastern black nightshade to nicosulfuron has been linked to the negligible translocation of these herbicides out of the treated leaf (Walker et al. 1994, Carey et al. 1997).

CGA 362622 metabolism. TLC analysis of treated leaf extracts of cotton, spurred anoda, and smooth pigweed revealed the presence of four major radioactive bands (Table 6.2). The major band with an Rf value of 0.6 corresponded to the parent herbicide, since it co-migrated with the standard ^{14}C-CGA 362622 on the TLC plates. Three unknown metabolites with Rf values of 0.24, 0.42, and 0.89 were recovered from all species. The amount of radioactivity remaining at the origin was negligible.

Metabolism was rapid in cotton at both growth stages compared to the two weed species (Table 6.2). At 6 HAT, 69 to 70% of parent molecule remained in cotton. By 72 HAT, 62% of parent remained in two- to three-leaf cotton, but only 51% remained in cotyledon to one-leaf cotton. In comparison, at 6 HAT, 82% of parent remained in spurred anoda and 85% remained in smooth pigweed. At 72 HAT, 72% of parent remained in spurred anoda and 68% remained in smooth pigweed.

The predominant metabolite detected in all species has a Rf of 0.89 (Table 6.2). The amount of ^{14}C corresponding to this metabolite ranged from 23 to 28% in cotton at both growth stages and all exposure times. In contrast, this metabolite accounted for 12 to 14% of recovered ^{14}C in spurred anoda and 10% of ^{14}C in smooth pigweed. The levels of the other two metabolites (Rf values of 0.24 and 0.42) were low and similar in all plant species at all exposure times. However, at 48 and 72 HAT, the metabolite with Rf value of 0.42 accounted for 12% of the recovered ^{14}C in cotyledon cotton. The chemical identity and biological significance of the three metabolites of CGA 362622 is currently unknown.

The metabolism results of this study are summarized in Figure 6.3, which shows the fate of the parent herbicide in cotton and the two weeds as well as the formation of metabolites as a function of exposure time. It is again evident that the parent herbicide is metabolized more rapidly in cotton at both growth stages than the two weed species. Recently, Askew and Wilcut (2002) reported rapid metabolism of CGA 362622 in cotton and jimsonweed, but slower metabolism in sicklepod and peanut. The half-life of CGA 362622 in cotton was reported to be 0.8-d (Askew and Wilcut 2002). This rate of CGA 362622 metabolism in cotton is faster than

that observed in our studies. This difference is probably due to varietal differences. Also, Askew and Wilcut (2002) did not spray cotton seedlings with sublethal rates of formulated CGA 362622 prior to treatment with ^{14}C-CGA 362622.

Other investigators have reported variable metabolism rates of ALS inhibitors by smooth pigweed and spurred anoda. At 72 HAT, ALS-sensitive smooth pigweed metabolized 48% of chloransulam-methyl (Poston et al. 2002), 70% of chlorimuron (Manley et al. 1999), and 34% of nicosulfuron (Manley et al. 1999). Metabolism of thifensulfuron in spurred anoda also has been documented to proceed slowly with only 41% of thifensulfuron metabolized by 72 HAT (Walker et al. 1994). Also, hairy nightshade, eastern black nightshade, and black nightshade failed to metabolize 49 to 62% of rimsulfuron by 48 HAT (Ackley et al. 1999). Eastern black nightshade has also been reported to metabolize only 30% of nicosulfuron by 72 HAT (Carey et al. 1997).

The results of the present study demonstrate that differential absorption, translocation, and metabolism contribute to the observed selectivity of the herbicide CGA 362622. Low absorption, limited translocation and rapid metabolism of CGA 362622 appear to contribute to the tolerance of two- to three-leaf cotton to this herbicide. Likewise, limited translocation and rapid metabolism may account for the tolerance of cotyledon to one-leaf cotton to CGA 362622. The moderate tolerance of spurred anoda to this herbicide is most likely due to limited translocation. Finally, the susceptibility of smooth pigweed to CGA 362622 appears to be linked to the high rates of symplastic translocation and the slow metabolism of this herbicide in this weed species. By 72 HAT, smooth pigweed was showing epinastic symptoms typical of ALS inhibitor application, while cotton and spurred anoda plants did not show these symptoms. Differential affinity of the ALS enzymes from the three plant species for CGA 362622 is also possible and needs to be examined in the future.

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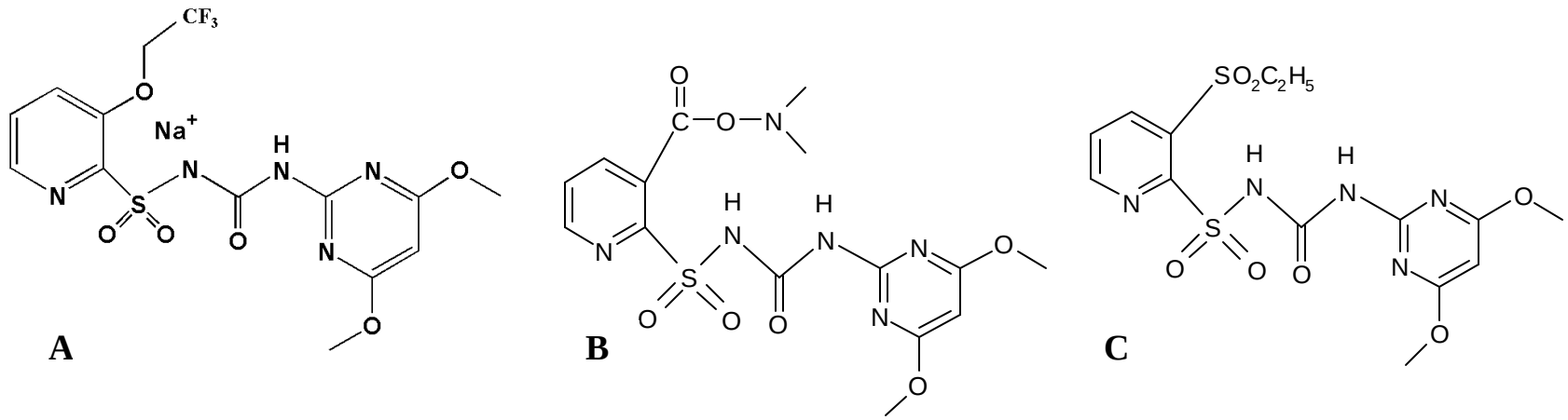

Figure 6.1. Chemical structures of A) CGA 362622, B) nicosulfuron, and C) rimsulfuron.

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Table 6.1. Absorption and translocation of radioactivity from foliar-applied ^{14}C-CGA 362622 in cotton (*Gossypium hirsutum*), spurred anoda (*Anoda cristata*), and smooth pigweed (*Amaranthus hybridus*).[a]

Species (Stage of growth)	Exposure time	Absorption	Distribution of absorbed radioactivity[b]				
			Treated leaf	Leaves below	Leaves above	Stem	Root
	hr	% of applied	%				
Cotton	6	24	96	1	1	1	0.5
(Cotyledon to one-leaf)	24	33	93	2	1	3	0.8
	48	59	95	1	1	2	0.5
	72	54	95	1	1	2	0.5
Cotton	6	24	95	1	1	2	1.2
(Two- to three-leaf)	24	22	94	1	1	3	0.9
	48	39	95	1	1	2	0.5
	72	30	95	1	1	2	0.9
Spurred anoda	6	42	80	1	7	7	3.2
	24	41	88	1	4	4	2.6
	48	55	96	1	1	1	1.0
	72	47	94	2	2	1	0.8
Smooth pigweed	6	55	30	12	20	37	1.5

	24	51	33	4	31	30	1.3
	48	57	57	1	25	16	0.9
	72	47	40	22	17	20	1.0
LSD (0.05)[c]		21	23	11	14	10	1.4

[a] All treatments contained 0.25% (v/v) nonionic surfactant.

[b] Means are the average of three studies.

[c] Means were separated using Fisher's protected LSD at the 0.05 significance level.

Table 6.2. Metabolism of foliar-applied ^{14}C-CGA 362622 in treated leaf extracts of cotton (*Gossypium hirsutum*), spurred anoda (*Anoda cristata*), and smooth pigweed (*Amaranthus hybridus*) at 6, 24, 48, and 72-h after treatment.[a,b]

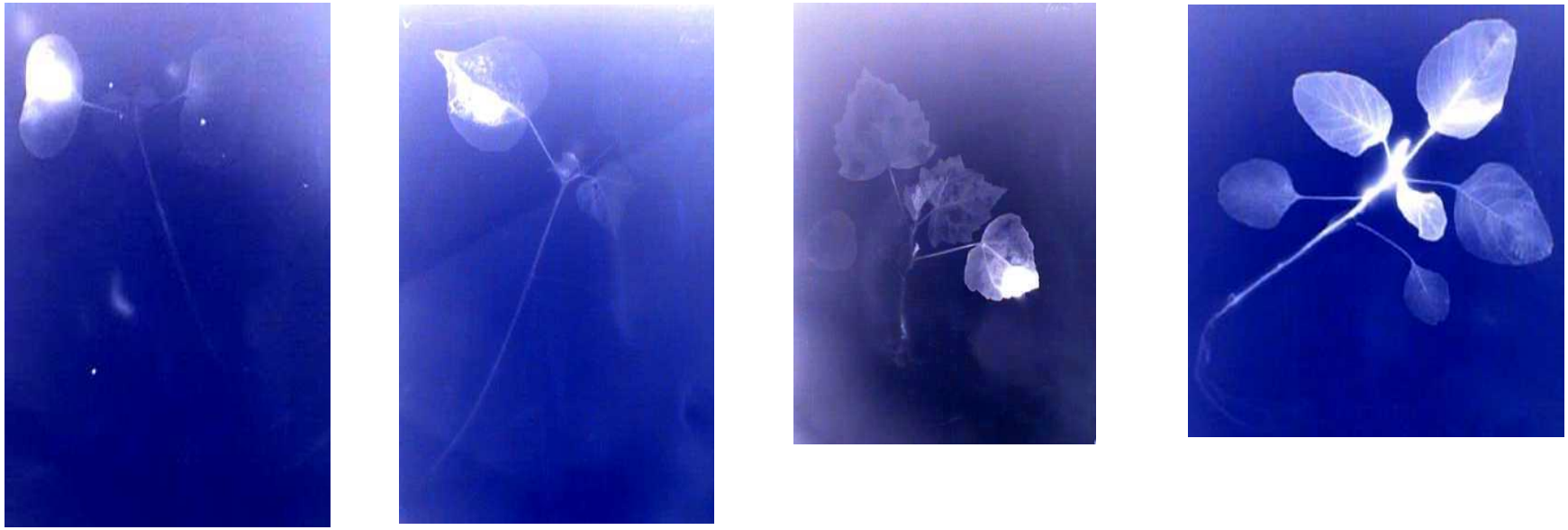
Species (Stage of growth)	Time after treatment	R_f values of detected metabolites[c]			
		0.24	0.42	0.6	0.89
	— h —	% of ^{14}C absorbed			
Cotton (Cotyledon to one-leaf)	6	2	2	69	25
	24	3	5	63	28
	48	4	12	55	26
	72	6	12	51	27
Cotton (Two- to three-leaf)	6	2	2	70	24
	24	2	4	67	25
	48	3	7	65	23
	72	3	7	62	23
Spurred anoda	6	2	2	82	12
	24	2	6	74	14
	48	2	8	74	13
	72	4	8	72	14
Smooth pigweed	6	2	2	85	10
	24	4	3	81	10
	48	5	5	77	10
	72	7	9	68	10
LSD (0.05)[d]		2	4	9	7

[a] All treatments contained 0.25% (v/v) nonionic surfactant.

[b] Means are the average of three studies.

[c] Standard ^{14}C-CGA 362622 migrated to an R_f value of 0.6.

[d] Means were separated using Fisher's protected LSD at the 0.05 significance level.



A **B** **C** **D**

Figure 6.2. Negative image of autoradiograph of A) cotyledon to one-leaf cotton, B) two- to three-leaf cotton, C) spurred anoda, and D) smooth pigweed harvested 72-h after treatment.

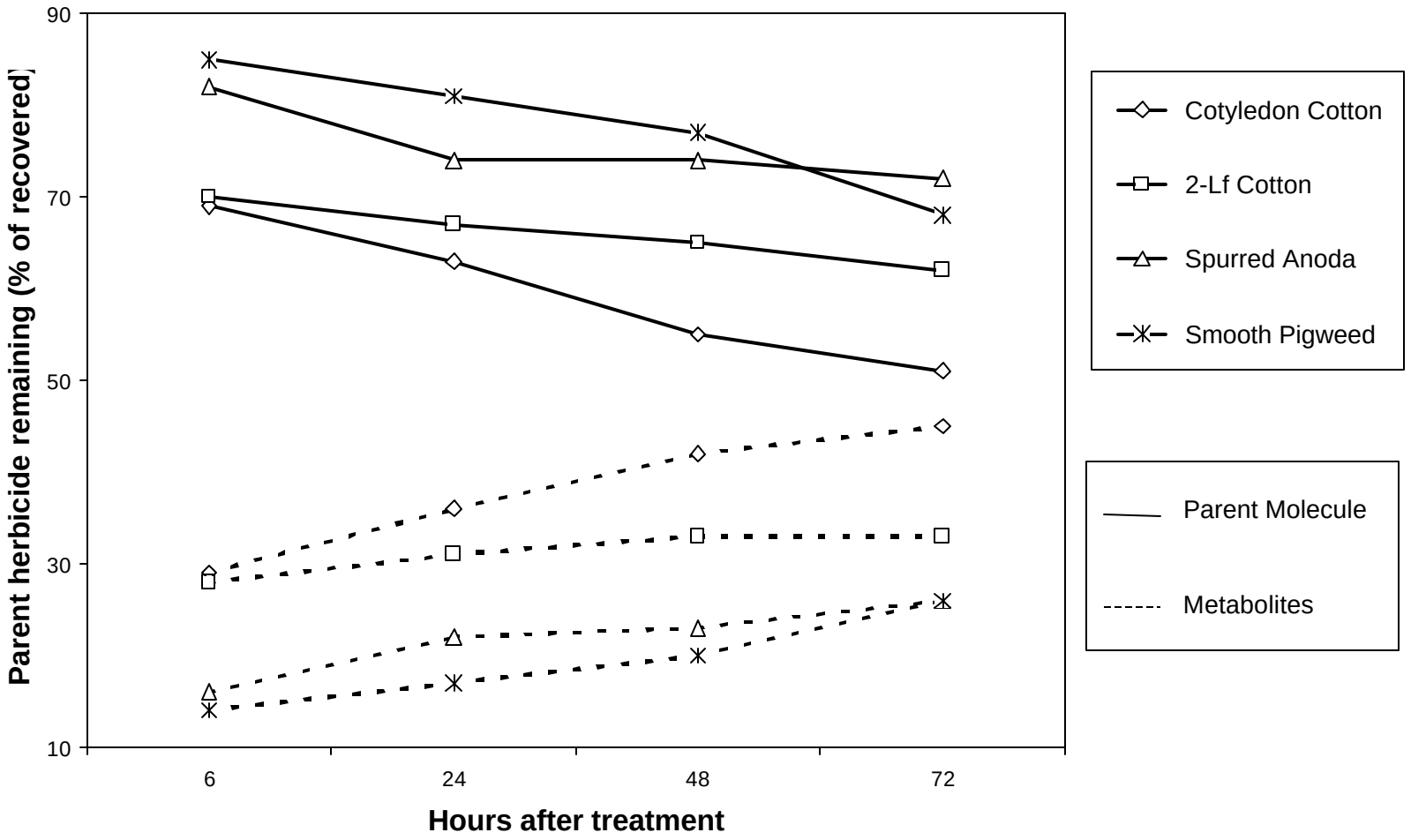


Figure 6.3. Metabolism of parent molecule (Rf = 0.6) and formation of metabolites in the treated leaves of cotyledon to one-leaf cotton (*Gossypium hirsutum*), two- to three-leaf cotton, spurred anoda (*Anoda cristata*), and smooth pigweed (*Amaranthus hybridus*). The LSD value compares means across species and time (P = 0.05).