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Fate of Diflubenzuron in Cotton, Soil, and Rotational Crops

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¹⁴C-labeled diflubenzuron (*N*-[[4-(chlorophenyl)amino]carbonyl]-2,6-difluorobenzamide) was applied to field-grown cotton either as a single dose (100 µg/leaf) for studies of absorption and metabolism or six and ten times (each treatment at a rate equivalent to 70 g of AI in 9.35 L of crop oil and 93.5 L of water/ha) for studies of radioactive residues in different parts of cotton plants at harvest and in soil and rotational crops. In cotton leaves treated with a single dose, there was little (<5%) photodegradation of diflubenzuron on the surfaces or metabolism within cotton leaves through 4-weeks posttreatment. The chemical was poorly absorbed (accumulation in leaves of <7% in 3–4 weeks), and losses to volatilization or weathering were minimum (<50% in 4 weeks) in samples that were not exposed to rain. Radioactive residues were low in mature seeds of cotton treated six or ten times with ¹⁴C-labeled diflubenzuron (<0.01 and ca. 0.02 ppm, respectively) and, although detected, were generally low in rotational crops, especially in edible portions. Postharvest residues of diflubenzuron in soil were persistent during the subsequent winter and spring months, but declined rapidly with the onset of high summer temperatures.

The insect growth regulator diflubenzuron [Dimilin, TH-6040, *N*-[[4-(chlorophenyl)amino]carbonyl]-2,6-difluorobenzamide] has promise for use in controlling a variety of arthropod pests of plants and animals (see reviews by Ivie, 1978, and Ivie and Wright, 1978). However, the greatest potential of diflubenzuron may be for use as an essential component of the proposed program for the eradication of the boll weevil, *Anthonomus grandis* Boheman. Moore and Taft (1975) first reported that diflubenzuron was highly effective in preventing the hatch of eggs from adult boll weevils treated with the material in the laboratory. Subsequently, Taft and Hopkins (1975) in small field-plot tests, and Lloyd et al. (1977) in large-scale field tests, demonstrated that properly timed applications of the chemical gave excellent control of field populations of this important pest. These findings, along with evidence that diflubenzuron is relatively nondestructive of field populations of beneficial species associated with cotton (Ables et al., 1977), and the inherent

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low mammalian toxicity, tend to encourage the commercial development of this insecticide.

The fate of diflubenzuron in a model ecosystem (Metcalfe et al., 1975), in mammals (Ivie, 1978), in boll weevils (Still and Leopold, 1975), and in insect pests of livestock (Ivie and Wright, 1978) has been reported. Because diflubenzuron may eventually be applied to large acreages of cotton, the present study was undertaken to determine its fate after single and multiple applications to cotton and in the soil and rotational crops subsequently grown on diflubenzuron-treated areas.

MATERIALS AND METHODS

Chemicals. Radiolabeled diflubenzuron, formulated as a 25% wettable (WP) or as the technical crystalline material (particle size of active ingredient was 2–5 µm), was supplied by the Thompson-Hayward Chemical Co., Kansas City, Kan. The molecule was uniformly radiolabeled with ¹⁴C in each of the ring moieties: 51.1% of the total radioactivity was in the difluorobenzoyl ring and 48.9% in the chlorophenyl ring; specific activity was 17.4 mCi/mmol. Radiochemical purity of both formulations was >99.0%, as indicated by thin-layer chromatography (TLC) and radiometric analyses. Also available were samples of nonradiolabeled technical diflubenzuron and a 25% WP formulation of the chemical, as well as the ten theoretical metabolites described by Ivie (1978) and listed

Table I. Chromatographic Behavior of Diflubenzuron and Certain of Its Theoretical Metabolites

Compound	R_f in solvent mixture ^a	
	A	B
4-Chlorophenyl urea	0.17	0.41
2,6-Difluorobenzoic acid	0.28	0.27
2,6-Difluorobenzamide	0.39	0.45
4-Chloroacetanilide	0.41	0.53
4-Chloro-3-hydroxydiflubenzuron ^b	0.57	0.44
4-Chloro-2-hydroxydiflubenzuron ^b	0.61	0.48
4-Chlorophenol	0.73	0.58
4-Chloroaniline	0.76	0.77
Diflubenzuron	0.81	0.81
4-Chloro-N-methylaniline	0.86	0.88
4-Chloro-N,N-dimethylaniline	0.90	0.92

^a A = 90:30:1, benzene-dioxane-acetic acid; B = 10:2:1, benzene-methanol-diethylamine. ^b Hydroxylation at the 3 or 2 position of the 4-chlorophenyl ring moiety of the diflubenzuron molecule.

in Table I. A crop oil (Sun-7N containing 2% of the emulsifier T-MULZ-A02) was obtained from Thompson-Hayward Co. for use in some of the field applications.

Fate on Field-Grown Cotton. The ¹⁴C-labeled diflubenzuron (WP) was diluted with four parts of the nonradiolabeled 25% WP formulation and then mixed with water to form an aqueous suspension having a concentration of 1000 ppm active ingredient (AI). This suspension was applied in situ to fully expanded leaves of field-grown "Stoneville 213" variety cotton; a 100- μ L aliquot (100 μ g of AI) was spread uniformly over the upper surface of each leaf with a micropipet.

At specified times posttreatment, three leaves were collected at random from different plants and processed immediately. Unabsorbed (external) radioactive material was recovered by rinsing the leaves thoroughly with methanol, and absorbed (internal) radioactive material was extracted by homogenizing the rinsed leaves with a Tissuemizer (Tekmar Co., Cincinnati, Ohio) in acetone and water (9:1, v/v). Solids separated by centrifugation were reextracted twice with acetone and then dried at 40 °C.

Combined solvent extracts of each sample were radioassayed by conventional liquid scintillation counting and then evaporated under vacuum to a convenient volume and analyzed with TLC by two-dimensional development on precoated glass plates (Silica gel 60, F-254, Brinkmann Instruments, Houston, Tex.) with solvent mixtures: (A) benzene-dioxane-acetic acid (90:30:1 v/v; Metcalf et al., 1975) and (B) benzene-methanol-diethylamine 10:2:1 v/v). Analytical standards cochromatographed with different samples were visualized under ultraviolet light; radioactive spots were located by autoradiography with x-ray film and then recovered and quantified by liquid scintillation counting.

The radiocarbon content of dried, extracted leaf tissues (100 mg/sample) was determined with combustion by the methods of Ivie (1978). (In these and all other studies involving combustions, comparable untreated control samples were processed and analyzed concurrently with the same procedures. Appropriate corrections were made for the natural background radioactivity.)

Persistence on Greenhouse-Grown Cotton Leaf and Silica Gel Surfaces Exposed to Sunlight. Potted plants from a greenhouse were also used to evaluate the persistence of diflubenzuron on cotton foliage. A ¹⁴C-labeled diflubenzuron formulation was prepared containing 500 ppm of diflubenzuron (25% WP) in a 1:4 emulsion of crop oil and water. Individual leaves were treated with 100 μ L of this preparation, which was spread over the

upper surface as evenly as possible with a microsyringe. The plants were then held outdoors in an unshaded area for periods up to 28 days. At the specified intervals, treated leaves were rinsed thoroughly with methanol. The methanol rinses were then analyzed as described, and radioactivity remaining in the rinsed leaves was determined by oxygen combustion.

A separate study was made of the persistence and degradation of diflubenzuron on silica gel chromatoplates (Eastman Chromagram sheets, Eastman Kodak Co., Rochester, N.Y.; 0.1 mm gel thickness, without fluorescent indicator). For this experiment, 200- μ g deposits of diluted technical grade ¹⁴C-labeled diflubenzuron (680 dpm/ μ g) were applied to 2.5 \times 20 cm strips of the silica gel sheets via 50 μ L of acetone solution. The treated strips were mounted on plywood and exposed outdoors to sunlight for as long as 28 days. Samples were analyzed at the specified intervals by scraping and extracting the gel with methanol and then quantitating the radiocarbon in the extracts and residual gel by liquid scintillation counting. The nature of the radiocarbon in the extracts was determined by two-dimensional TLC.

Both the treated potted cotton plants and the silica gel samples were usually placed in a protected area at night and were protected against occasional rainfall during the day. However, the plants were inadvertently exposed to ca. 1 cm of rain between the third and fourth weeks of the study. In both studies, two or three replicates were run at each analysis interval.

These studies with the potted cotton plants and silica gel plates were designed primarily to evaluate potential photodegradation of diflubenzuron. The work with cotton described in this section differed from that involving single applications to field-grown cotton primarily in that the potted plants were protected from moisture and the formulations used in the treatments were made with crop oil.

Tests of Multiple Applications of Diflubenzuron. Residues in Cotton Plants. Two plots of field-grown "Stoneville 213" cotton, each 1.7 m² in size, were enclosed in a 1.8 \times 1.8 \times 5.5 m long cage covered with plastic screen to exclude phytophagous pests. The plants were sprayed with an aqueous suspension of ¹⁴C-labeled diflubenzuron (25% WP, diluted 20-fold with nonradioactive diflubenzuron) and crop oil at rates equivalent to 70 g of AI in 9.35 L oil and 93.5 L water/ha. Treatments were initiated July 23, 1976, when all plants were well into the fruiting phase of development and were continued at ca. 5-day intervals until one plot had been treated six times (Aug 18) and the other ten times (Sept 4). No bolls were open during the first six applications, but ca. 10–20% were open after ten applications. (Hereafter, these treated plots are identified as 6A and 10A.)

All seed cotton in the 10A plot that had been exposed directly to the spray was collected at the conclusion of the scheduled treatments and held separately for processing. Plants were defoliated at the customary time, and all leaves on the ground or remaining on the plants were collected Sept 23. Later, on Oct 18, new growth leaves, seed cotton, and cotton stalks with their roots were collected and held separately. Seeds (separated by hand) and lint were not dried, but all other plant materials were dried 48 h at 40–50 °C. The leaves and woody parts of plants were weighed and ground separately in a Wiley mill to pass a 20-mesh screen. Each milled sample was mixed thoroughly and held for further analysis.

Random samples consisting of aliquots (100 mg) of milled plant materials, whole or separated seeds (both

nondelinted and acid-delinted), and lint (100 mg) were combusted and radioassayed by liquid scintillation counting.

Residues in Soil. Cores (2.2 × 22.9 cm deep) of soil (Lufkin fine sandy loam; see Bull et al., 1970, for properties) were taken at three to five random locations on a diagonal line across each plot. Each core was divided into three or more subsamples according to depth. Samples analyzed by combustion were dried 24 h at 40 °C; those subjected to solvent extraction and analysis with TLC were not dried.

For combustion analyses of dried soil, three or more 1-g aliquots of each sample were weighed into ceramic boats and combusted individually in an oxygen atmosphere at 1000 °C in a Lindberg furnace. The combustion gases were passed through a gas scrubber submerged in 20 mL of a 1:1 mixture of Carbo-Sorb and liquid scintillation fluid for 10 min, and then the radioactivity in the trapping solution was counted. Appropriate standards were processed concurrently, and corrections were made for combustion efficiency, background, and quenching.

Some soil samples (100 g each) were collected from the surface of the 10A plot at two different times and extracted successively with ethyl acetate (3:1 v/w), acetone (2:1), and methanol (2:1) and then twice with a 19:1 mixture of acetone and water (2:1). Solvent extracts and soil were separated via centrifugation; the extracts were radioassayed and then filtered, evaporated to a convenient volume, and analyzed with TLC as described. Extracted soil was dried and analyzed by combustion to determine unextracted radiocarbon.

Mass spectral analysis was used to confirm the chemical identity of diflubenzuron present in one of the soil extracts. About 100 µg of diflubenzuron was extracted from a soil sample collected on April 7, 1977, approximately 6 months after reincorporation of the crop residues into the soil. After cleanup by TLC and high-performance liquid chromatography (Oehler and Holman, 1975), the sample was analyzed by direct insertion probe mass spectrometry (Ivie, 1978).

Residues in Rotational Crops. Except for the lint and seeds, most of the milled cotton plant materials (90% of total) was distributed uniformly over the surface area of the respective treated plots on Nov 1, 1976, and cultivated thoroughly into the top ca. 10 cm of soil. After ca. 3 weeks during which ca. 7.6 cm of rain fell, soil cores were collected for residue analyses; then each plot was planted, half with wheat seed and half with collard seedlings. Later, in the spring of 1977, radish and pinto bean seeds were planted. Samples of each of these rotational crops and of native grasses and weeds growing in the plots were collected at appropriate intervals and were processed and analyzed by combustion to determine residues of radiocarbon.

RESULTS

Fate after Treatment of Field-Grown Cotton. After a single foliar application to field-grown cotton, residues of ¹⁴C-labeled diflubenzuron persisted tenaciously on leaf surfaces. After 14 days, almost 90% of the applied dose was unabsorbed; only ca. 4.8% was recovered in internal extracts, and <1% could not be extracted from leaf tissues (Table II). After 21 days and following a heavy rainfall, ca. 23% of the applied diflubenzuron still remained on the treated leaf surfaces.

Analyses of all extracts with two-dimensional TLC and autoradiography showed only the parent compound.

Persistence on Greenhouse-Grown Cotton Leaves and Silica Gel Plates. A formulation of ¹⁴C-labeled diflubenzuron (25% WP) in oil and water was also quite

Table II. Fate of Foliar Applied ¹⁴C-Labeled Diflubenzuron on Individual Cotton Leaves in the Field (100 µg/Leaf)

Days posttreatment	% of dose in indicated fraction ^a			
	External rinse	Internal extract	Unextractable	Lost
0	98.2	1.8	0.0	0.0
1	89.6	2.8	0.1	7.5
3	88.6	2.5	0.1	8.8
7	89.2	2.2	0.1	8.5
14	87.4	4.8	0.5	7.3
21 ^b	22.7	6.8	0.4	70.1

^a Two-dimensional TLC indicated only diflubenzuron in all external or internal extracts. Data are averages of six replicates. ^b Ca. 7.6 cm of rain fell on treated plants between 14 and 21 days posttreatment.

Table III. Persistence of ¹⁴C-Labeled Diflubenzuron on Leaves of Potted Cotton Plants and Silica Gel Surfaces Exposed to Sunlight

Radioactive fraction	% of dose at indicated days posttreatment			
	0	7	14	28
Cotton leaf				
Methanol wash	97.7	78.6	64.5	55.7
Leaf residue	2.3	6.8	5.9	6.8
Lost	0	14.6	29.6	37.5
Silica gel plate				
Methanol extract	99.7		70.9	46.4
Gel residue	0.3		0.6	0.8
Lost	0		28.5	52.8

persistent on the surfaces of cotton leaves. After 28-days exposure to summer sunlight (and protection against rainfall), more than half the applied diflubenzuron was still unabsorbed and only ca. 7% was associated with the leaf residue (Table III). The remainder (38%) was presumably lost through volatilization or other "weathering" factors.

Technical grade ¹⁴C-labeled diflubenzuron was also persistent when applied to thin layers of silica gel and exposed to sunlight. About 70% of the applied radiocarbon was recovered from the gel after 2 weeks of exposure, and after 4 weeks, slightly less than half remained on the gel (Table III). Only low quantities of radioactive material were not extracted from the gel with methanol; that not accounted for probably represents both volatilization of the applied ¹⁴C-labeled diflubenzuron and weathering of the gel itself.

Two-dimensional TLC analyses of the extracts from cotton leaves and from silica gel revealed that diflubenzuron on such surfaces is highly resistant to sunlight photodegradation. In all extracts, unchanged diflubenzuron comprised >98% of the total radiocarbon present. No degradation products were observed with sufficient consistency or in sufficient quantity to permit chemical or chromatographic analysis. Although the radiocarbon not extracted from the leaf or gel residue may represent in part products of diflubenzuron degradation, no attempts were made to study the chemical nature of the low levels of radioactivity in these fractions.

Radiocarbon in Cotton Plants after Multiple Applications of Diflubenzuron. The results of combustion analyses indicated there was little accumulation of radiocarbon in the seed collected from either the 6A or 10A plots of cotton (Table IV). Less than 0.01 ppm (the lower limit of reliable sensitivity of the radioassay procedure) were found in seeds of plants in 6A plots and a maximum of ca. 0.02 ppm in those from 10A plots. However, radioactive residues were relatively high in some parts of the plants, particularly in the leaves that were on the plants

Table IV. Radioactive Residues in Cotton Plants Treated in the Field with Spray Applications of ¹⁴C-Labeled Diflubenzuron

Plant sample	Total dry weight, g	Ppm (dry wt) ¹⁴ C-labeled diflubenzuron equivalents (\pm SE) ^a
Six Applications		
Seed (AD) ^b		
Whole		<0.01
Hulls		<0.01
Meat		<0.01
Seed (ND) ^c		
Whole	196	<0.01
Hulls		<0.01
Meat		<0.01
Leaves, old ^d	253	37.62 \pm 3.93
Leaves, new ^e	15	0.60 \pm 0.30
Stalk, root, and calyx	310	0.76 \pm 0.12
Total residue ^f		17.22 \pm 4.61
Lint	118	0.23 \pm 0.40
Ten Applications		
Seed (AD)		
Whole, A ^f		0.02 \pm 0.01
Whole, B ^g		<0.01
Hulls, A		<0.01
Hulls, B		0.02 \pm 0.01
Meat, A		<0.01
Meat, B		<0.01
Seed (ND)		
Whole, A	107	0.02 \pm 0.00
Whole, B	57	0.02 \pm 0.02
Hulls, A		<0.01
Hulls, B		0.01 \pm 0.01
Meat, A		<0.01
Meat, B		<0.01
Leaves, old ^d	328	106.91 \pm 17.38
Leaves, new ^e	28	1.38 \pm 0.64
Stalk, root, and calyx	288	3.29 \pm 2.21
Total residue ^f		50.90 \pm 8.32
Lint, A	65	0.52 \pm 0.44
Lint, B	36	0.31 \pm 0.20

^a Each combustion analysis was replicated at least three times. ^b AD = acid delinted. ^c ND = nondelinted.

^d Leaves on the plant during treatment. ^e New growth posttreatment and postdefoliation. ^f "A" samples are bolls opening posttreatment. ^g "B" samples are bolls open during the treatment period. ^h Mixture of milled old and new leaves, stalk, and root recombined on the basis of proportional weight.

during treatment. In all cases, higher levels of radioactive residues were found in samples from plants treated ten times. It is noteworthy that new leaves, which developed after treatments ceased, contained appreciable levels of radiocarbon (ca. 0.60 ppm in 6A plots, 1.38 ppm in 10A plots). This indicates there was translocation of diflubenzuron and/or products of its decomposition from the woody portion of the plant into new foliar growth.

Attempts to determine the identity of the low levels of radioactivity in new growth were unsuccessful. However, when aliquots of the dried and milled woody portions of plants from 10A plots were extracted as described and analyzed, ca. 73% of the total radioactivity was recovered in the solvent fraction; all the extracted radioactivity was associated with a single compound that cochromatographed with diflubenzuron in two-dimensional TLC.

Radiocarbon in Rotational Crops. Radioactive residues were found in all plants subsequently grown in the two plots used for treatments of cotton (Table V). As in the cotton plants, radiocarbon levels were generally higher in the rotational crops in the 10A plot. However, in both plots, residues in the edible portions were low. For example, in the 6A plot, collard greens contained only ca.

0.04 ppm diflubenzuron equivalents, and immature and mature beans and wheat seed had no detectable residues. In the 10A plots, collards contained ca. 0.09 ppm and mature beans ca. 0.04 ppm, but wheat seed and immature beans had no significant radioactive residues. The highest levels of radioactivity in plants from both plots were found in native grass, but attempts to characterize this residual radioactivity were unsuccessful.

Radiocarbon in Soil. The results of combustion analyses of the soil collected from the 6A and 10A plots (Table VI) indicated that most of the radiocarbon was concentrated in the upper 7.5-cm layer of soil and that levels of radioactivity were generally higher in the 10A plots. Results of analyses of soil from the 10A plot on the later dates suggested that the radioactivity was tending to migrate to greater depths (7.5 to 15.0 cm). However, it should be noted that there was considerable variation among residues measured at different sample sites, and the latter result may have been caused mechanically by cultivation rather than by leaching.

During the first 6 months after reincorporation of treated plant materials into the soil, it was apparent that radioactive residues did not dissipate to any appreciable extent; however, later analyses (July 3 and Sept 12, 1977) provided evidence that these levels were declining in both plots.

Other combustion analyses of soil collected April 29, 1977, from the 10A plot (not shown in Table VI) demonstrated that the relative distribution of total radiocarbon (average of four core samples) in sequential 2.5-cm increments from the surface to a depth of 12.5 cm was 40.8 \pm 13.3, 30.0 \pm 7.3, 17.4 \pm 10.1, 10.7 \pm 8.6, and 3.1 \pm 2.0%, respectively. Plainly, most of the radioactivity was located in the top 10 cm of soil, but there was substantial variation across that layer at the different sampling locations. This variation was probably a result of the nonuniform distribution of treated plant materials when they were reincorporated into the soil.

The nature of the radioactivity associated with treated soil was determined by exhaustive extraction with different solvents and by TLC analysis of samples taken from the surface (0–5 cm) of the 10A plot. Tests of soil collected April 7, 1977, revealed that most (ca. 87%) of the radioactive material could be recovered by solvent extraction. Of the total, 81% was identified as diflubenzuron on the basis of TLC and mass spectral analysis; the balance represented small quantities of two unknowns (ca. 2% each), a product (1.7%) that cochromatographed with the 4-chlorophenylurea standard, and 13.2% that could not be extracted. Similar analyses of soil collected on Sept 12, 1977, indicated that 79% of the total radiocarbon was extracted and all of this cochromatographed with diflubenzuron on TLC.

DISCUSSION

The experiments reported here demonstrated that, under conditions approximating anticipated normal use in cotton production, diflubenzuron is persistent. After foliar application, diflubenzuron adheres well to leaf surfaces; it is absorbed very slowly and is not lost rapidly by volatilization. However, heavy rainfall during some of the studies did cause an appreciable reduction in the residues on surfaces of cotton foliage. Diflubenzuron was also highly resistant to decomposition on cotton plants, both to photodecomposition on leaf surfaces and to metabolism after penetration into leaves.

After multiple treatment of cotton with sprays of ¹⁴C-labeled diflubenzuron, we found high levels of radioactive residues in vegetative growth, but only minimum residues

Table V. Radioactive Residues in Rotational Crops Planted in Plots Treated with ¹⁴C-Labeled Diflufenuron during the 1976 Cotton Season^a

Plant	Harvest date	Weight, g		Ppm (dry wt) ¹⁴ C-labeled diflufenuron equivalents (±SE)
		Wet	Dry	
Six Applications				
Collards	3/1/77	99.2	19.0	0.04 ± 0.01
Native grass	3/1/77	99.8	19.3	0.11 ± 0.01
Native weeds	3/1/77	35.0	6.9	0.06 ± 0.02
Pinto beans ^b	4/5/77	23.2	3.9	0.03 ± 0.00
Pinto beans (pod) ^c	4/29/77	56.4}	17.9	< 0.01
Pinto beans (plant) ^c	4/29/77			0.02 ± 0.01
Pinto beans (seed) ^d	5/24/77	21.0}	14.2	< 0.01
Pinto beans (husk) ^d	5/24/77			0.03 ± 0.01
Pinto beans (plant) ^d	5/24/77	14.5	2.5	0.03 ± 0.00
Radish ^b	4/5/77	9.5	1.2	0.05 ± 0.01
Wheat (head) ^d	5/24/77	5.6	1.7	< 0.01
Wheat (plant) ^d	5/24/77	33.9	8.0	0.01 ± 0.00
Ten Applications				
Collards	3/1/77	189.2	29.6	0.09 ± 0.00
Native grass	3/1/77	236.8	47.5	0.17 ± 0.02
Pinto beans ^b	4/5/77	28.8	5.0	0.10 ± 0.00
Pinto beans (pod) ^c	4/29/77	67.6}	21.2	< 0.01
Pinto beans (plant) ^c	4/29/77			0.12 ± 0.01
Pinto beans (seed) ^d	5/24/77	21.7}	15.3	0.04 ± 0.01
Pinto beans (husk) ^d	5/24/77			0.11 ± 0.01
Pinto beans (plant) ^d	5/24/77	10.0	2.9	0.16 ± 0.01
Radish ^b	4/5/77	11.3	2.9	0.16 ± 0.01
Radish (top) ^d	5/17/77	120.2	9.8	0.09 ± 0.01
Radish (tuber) ^d	5/17/77	115.3	8.1	0.06 ± 0.01
Wheat (head) ^d	5/24/77	51.6	20.2	< 0.01
Wheat (plant) ^d	5/24/77	85.2	25.8	0.01 ± 0.00

^a These crops were planted after the reincorporation (on 11/23/76) of radioactive cotton plant residues into the soil. Each combustion analysis was replicated at least three times. ^b Plants immature, no fruiting forms. ^c Beans with immature pods; "plant" = balance remaining after pods removed. ^d Mature plants; wheat heads fully developed but not at harvest stage.

Table VI. Radioactive Residues in Soil Cores Taken from Plots Treated with ¹⁴C-Labeled Diflufenuron during the 1976 Cotton Season^a

Sample date ^c	Ppm ¹⁴ C-labeled diflufenuron equivalents (±SE) at indicated depth (cm) ^b					
	0-7.5		7.5-15.0		15.0-22.5	
	6 appl.	10 appl.	6 appl.	10 appl.	6 appl.	10 appl.
10/20/76	0.07 ± 0.02	0.12 ± 0.03	< 0.01	< 0.01	< 0.01	< 0.01
11/23/76	0.10 ± 0.02	0.30 ± 0.04	< 0.01	< 0.01	< 0.01	< 0.01
1/21/77	0.04 ± 0.01	0.29 ± 0.05	0.02 ± 0.01	0.05 ± 0.02	< 0.01	0.02 ± 0.01
4/29/77	0.05 ± 0.01	0.30 ± 0.06	< 0.01	0.14 ± 0.03	< 0.01	< 0.01
7/3/77	0.04 ± < 0.01	0.13 ± 0.01	< 0.01	0.03 ± < 0.01	< 0.01	< 0.01
9/12/77	0.02 ± < 0.01	0.10 ± 0.01	< 0.01	0.01 ± < 0.01	< 0.01	< 0.01

^a Multiple treatments were initiated July 23, 1976, and terminated either Aug 18 (6 appl.) or Sept 4 (10 appl.). ^b Data are averages of analyses of three to five core samples/plot; each combustion analysis was replicated at least three times. Sample sites were selected at random on a diagonal line across each plot. ^c Samples on 10/20/76 were collected when all plant material was removed from plots; subsequent samples were collected after reincorporation of treated plant materials (90%) in soil.

in mature seeds. Radioactive residues were taken up by rotational crops subsequently grown in the treated areas, but the levels in these crops were generally low, and they were often undetectable in those parts of the plants that are normally consumed.

In these tests, the soil of the treated plots was brought into contact with ¹⁴C-labeled diflufenuron in two ways, directly by runoff of sprays and indirectly when treated plants were milled and cultivated into the soil. Analyses of the treated soil demonstrated that diflufenuron was somewhat stable under the test conditions. There was no appreciable decline in total residues through ca. 9 months after the initiation of treatments, but analyses made after ca. 1 year indicated that the radiocarbon in soil was declining. This belated diminution of radioactivity from treated soil may have been caused by increased volatilization due to the high summer temperatures and by the greater accessibility of the diflufenuron molecule to

mechanisms of degradation as a result of the progressive decay of the plant material with which it was associated.

Although not specifically studied, the extent of leaching in soil seemed minimum; most radioactivity was consistently detected in the top 10-cm layer of soil where it was originally incorporated. There was some increased recovery of radioactivity at lower levels of soil in the plot that received ten applications of diflufenuron; however, these residues too were tending to decline in samples collected at the later dates.

The apparent stability of diflufenuron in soil was not unexpected. Metcalf et al. (1975) detected little decomposition of this chemical in a silty clay loam in the laboratory. Also, though other laboratory studies (Verloop and Ferrell, 1977) have shown that diflufenuron was almost totally decomposed after 4 weeks in a nonsterile sandy loam soil, there is evidence (Nye, 1977) that the chemical is persistent in treated leaf litter and other or-

ganic materials. Indeed, the major portion of the diflubenzuron applied to soil in our study was associated with treated plant materials and this may have provided some protection from decomposition. However, our method of treating the soil is comparable to that which will occur when the chemical is used for insect control on cotton.

As would be expected, the residue pattern was more favorable for the plot that received the fewest applications. Therefore, if the levels of residues observed in this study should subsequently be judged undesirable, some limitation might be placed on the number of diflubenzuron applications to be used in commercial practice.

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Pesticide Interactions in Oats (*Avena sativa* L. 'Neal')

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According to greenhouse studies on oats (*Avena sativa* L. 'Neal'), interactions occur between both alachlor (2-chloro-2,6-diethyl-*N*-(methoxymethyl)acetanilide) and trifluralin (α,α,α -trifluoro-2,6-dinitro-*N,N*-dipropyl-*p*-toluidine) and the photosynthesis inhibiting herbicides, atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine), cyanazine (2-[[4-chloro-6-(ethylamino)-*s*-triazine-2-yl]amino]-2-methylpropionitrile), diuron (3-(3,4-dichlorophenyl-1,1-dimethylurea), and methazole (2-(3,4-dichlorophenyl)-4-methyl-1,2,4-oxadiazolidine-3,5-dione). The effect on oat dry weights of photosynthesis inhibiting herbicides applied to the soil was attenuated by the application of alachlor or trifluralin to the soil. Leaf necrosis, attributable to photosynthesis inhibiting herbicides, was decreased with the application of alachlor or trifluralin. However, the effect of a foliar application of atrazine on oat dry weights was not attenuated by the soil application of alachlor or trifluralin. Neither alachlor nor trifluralin reduced the effects of the photosynthesis inhibiting herbicides on CO₂ fixation or on respiration in excised leaves. Application of alachlor, phorate (*O,O*-diethyl *S*-(ethylthiomethyl)-phosphorodithiate), and trifluralin in combination with ¹⁴C-labeled photosynthesis inhibiting herbicide decreased the root length and the amount of uptake of the ¹⁴C-labeled photosynthesis inhibiting herbicides. A high correlation was observed between root length and the percentage of ¹⁴C-labeled photosynthesis inhibiting herbicides taken up. These data show that alachlor and trifluralin inhibit root growth and thereby reduce absorption of the photosynthesis inhibiting herbicides.

The use of pesticides is common in today's agriculture. During the last decade, the use of combinations of two or more pesticides on the same crop has become prevalent in attempts to increase the number of pests controlled. When two pesticides are combined, three responses may occur: (1) they may act independently, (2) their combined

effects may be greater than the sum of the responses given by the individual treatments, or (3) their combined effects may be less than the sum of the responses given by the individual treatments.

The objectives of this study were to determine whether interactions between commonly used pesticides alter plant responses and then to explain possible relationships among the pesticides and the responses they evoke.

EXPERIMENTAL SECTION

A preliminary study was conducted to determine workable ranges of concentration for alachlor (2-chloro-2',6'-diethyl-*N*-(methoxymethyl)acetanilide), atrazine

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