Uptake, Distribution, and Metabolism of Monuron and Diuron by Several Plants

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Root uptake and distribution of ^{14}C-diuron from solution culture by soybean (susceptible) and cotton (tolerant) indicated that neither differential absorption nor distribution of the herbicide could account for the 10-fold difference in ED_{50} value. Accumulations of 1-(3,4-dichlorophenyl)-3-methylurea (phytotoxic) and 1-(3,4-dichlorophenyl)urea (nonphytotoxic) occurred, respectively, in the leaves of soybean and cotton. Diuron was identified in soybean leaves but not in cotton leaves. Accumulations of 1-(*p*-chlorophenyl)-3-methylurea and 1-(*p*-chlorophenyl)urea occurred, respectively, in the leaves of soybean and cotton grown in solutions containing ^{14}C-monuron. Labeled *p*-chloroaniline was detected in cotton but not in soybean. Most of the ^{14}C which accumulated in cotton, oat, and soybean leaves, after root absorption of the labeled herbicide, was not the parent compound. Corn leaves contained significant amounts of the parent herbicide. The tolerance of cotton was attributed to metabolism, but differences in susceptibility among oat, soybean, and corn appeared to be related more to differential absorption.

The phenylureas are rapidly absorbed by roots of plants and translocated acropetally in the transpiration stream (*2, 3, 8*), and absorption of labeled 3-(*p*-chlorophenyl)-1,1-dimethylurea (monuron) and 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron) by leaves and stems of plants has been shown (*2, 3, 5, 8*). Accumulation of ^{14}C in aerial portions of plants occurs after root absorption of these labeled herbicides and is dependent, to a large extent, on the rate of transpiration. Muzik, Cruzado, and Loustalot (*12*) and Minshall (*11*) have shown conclusively that factors favoring reduced transpiration delay or prevent phytotoxic symptoms in plants exposed to otherwise lethal concentrations of monuron. In work reported by Bayer and Yamaguchi (*2*) radioautographs of intact plants indicated no species differences in absorption, distribution, or accumulation of ^{14}C among barley, soybean, and red kidney bean plants exposed to ^{14}C-diuron in solution culture. Much of the work with ^{14}C-monuron and diuron utilized gross radioautography of plants and did not unequivocally demonstrate absorption, translocation, or distribution of the intact herbicide molecule.

The metabolic fate of these widely used herbicides is of obvious concern in a society so conscious of pesticide residues. Much remains to be learned in this area, but several important contributions have been made. Hill *et al.* (*9*) reported that certain soil microorganisms were capable of utilizing monuron as the sole carbon source. These workers observed about 10% loss of methyl-labeled ^{14}C-monuron as $^{14}CO_2$ in 90 days from soil containing 2 p.p.m. Several others have correlated disappearance of phytotoxic effects of these compounds from soils with factors favoring microbial growth.

Fang *et al.* (*5*) reported a monuron complex in the leaves of bean after foliar application of ^{14}C-monuron. The unidentified monuron complex increased with time and the parent herbicide decreased.

Sweetser (*14*) showed that monuron complexed with flavine mononucleotide and, upon illumination, was inactivated. Welker and Holm (*15*) noted modification of ^{14}C-monuron soon after treatment of seeds and seedlings of *Abutilon theophrasti* Medic. Three radioactive substances were found in extracts of seedlings 48 to 72 hours after growth began; but after 7 days, only two radioactive components were separated.

Dalton, Evans, and Rhodes (*4*) identified decomposition products of diuron in soil extracts from cotton fields treated with the herbicide, suggested a breakdown sequence, and gave the relative phytotoxicities of metabolites. The sequence of breakdown involved demethylation followed by hydrolysis of the resulting urea. DMU (diuron with one methyl group removed) was reported to be about one half as phytotoxic as diuron; the products formed by removing the second methyl group, 1-(3,4-dichlorophenyl)urea, and by hydrolysis of the urea, 3,4-dichloroaniline, were nonherbicidal. The same degradation sequence was found by Geissbühler *et al.* (*7*) for 3-(*p*-chlorophenoxy)-phenyl-1,1-dimethylurea (chloroxuron) in plants and soil microorganisms. Evidence suggests that microbial decomposition is the primary disposition process for phenylureas in soils.

The present research was conducted to compare phytotoxicities of monuron and diuron to four plant species, to follow the progressive uptake and distribution patterns of ^{14}C from labeled diuron in soybean and cotton, to study uptake and distribution patterns of ^{14}C in four species exposed to ^{14}C-diuron for a single time period, and to determine the metabolic fate of ^{14}C-monuron and ^{14}C-diuron in selected plants.

Methods and Materials

Seeds of oat (*Avena sativa* L., cv. Markton), cotton (*Gossypium hirsutum* L., cv. Coker), corn (*Zea mays* L., US-13 Hybrid), and soybean (*Glycine max* L., Merrill, cv. Lee) were planted in flats of autoclaved Lakeland sandy loam and allowed to germinate and grow in the greenhouse for 4 days. Seedlings were washed free of soil particles and transferred to nutrient culture solutions for 2 to 4 days before addition of herbicides. All

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experiments were conducted in the greenhouse without aeration. In preliminary tests, aeration had no apparent effect on uptake and translocation of ^{14}C-diuron.

The culture solution was similar to that described by Hoagland and Arnon (*10*) except that ferric ethylene-(dinitrilo)tetraacetic acid (EDTA, 8.0 p.p.m. concentration of Fe) was substituted for ferric tartrate. To exclude light, glass jars containing the solutions were covered with aluminum foil, and the tops were fitted with paraffin-covered corks.

The phytotoxicities of monuron and diuron were determined by treating week-old seedlings (three seedlings per jar) in jars containing several concentrations of the herbicides in 400 ml. of culture solution. After 11 days of exposure, fresh weights of tops were recorded and used as a measure of phytotoxicity. ED_{50} values (molar concentrations required to reduce fresh weights 50%) were obtained from plots of weight (per cent of control) *vs.* concentration.

Carbon-14–diuron labeled in carbonyl carbon (specific activity 0.96 mc. per mmole) and ^{14}C-monuron labeled uniformly in the ring (specific activity 0.38 mc. per mmole) were used. The sample of ^{14}C-diuron contained 4.0% of the monomethyl derivative of diuron and that for ^{14}C-monuron 2.6% of its monomethyl derivative. No attempt was made to remove these impurities. Isotope studies were conducted in glass jars with two seedlings in 100 ml. of culture solution per jar. Solutions containing labeled herbicide were prepared and the seedlings were transferred immediately. All ^{14}C-diuron solutions were $1.07 \times 10^{-6} M$ and all ^{14}C-monuron solutions $2.52 \times 10^{-6} M$.

Uptake and distribution of ^{14}C-diuron in cotton and soybean were determined after 12, 24, 48, 72, 96, and 120 hours' exposure. A separate culture for each species was radioassayed at each time interval. Two separate experiments were conducted. ^{14}C-monuron was included for the 120-hour time interval of the second experiment.

Uptake and distribution of ^{14}C-diuron by oat, soybean, corn, and cotton plants were studied in another experiment. Duplicate cultures and an exposure of 120 hours were employed. Otherwise, plant-culture procedures were identical to those described above.

Plants growing in the ^{14}C-labeled herbicide solutions were removed and the root systems rinsed with distilled water. The rinse water was returned to the culture jar and the solutions saved for ^{14}C determination. Roots were blotted lightly with tissue paper to remove excess water. The plants were sectioned into desired fractions (usually roots, stems, and leaves) and fresh weights of each recorded. The plant fractions were diced and transferred to a beaker containing 50 ml. of 95% ethanol. The beaker was placed in an 80° C. water bath for 5 minutes, then the extract was decanted, and 50 ml. of fresh alcohol were added to the beaker. The plant material was extracted a second time for 5 minutes, and the two extracts were combined. Extracted plant material was saved for determination of residual ^{14}C. Unless otherwise stated, cotyledons of soybean were harvested and extracted with the leaves.

Thin-layer chromatographic plates were prepared from silica gel impregnated with a fluorescence indicator (Merck HF_{254}). A paste, consisting of 30 grams of adsorbent and 68 ml. of H_2O, was spread to a thickness of 250 microns over the plate, and dried at 100° C. for 2 hours.

Aqueous solutions and alcohol extracts used for chromatography were evaporated under a gentle stream of air. The resulting residues were redissolved in 95% ethanol for spotting. Chromatographic systems employed were 2 to 1 benzene–acetone (v./v.) and 1 to 1 chloroform–ethyl acetate (v./v.). Chromatograms were radioautographed with Kodak No-Screen, medical x-ray film. Authentic labeled herbicides and nonlabeled intermediates (visualized with an ultraviolet lamp) were cochromatographed on each chromatogram for comparison with unknowns in plant extracts. Metabolites were tentatively identified by comparison of their R_f values with known compounds.

Carbon-14 in all aqueous solutions, alcohol extracts, and plant meals was counted with a thin-window, gas flow counter. The initial ^{14}C in culture solution of labeled herbicide was determined by counting six 1-ml. samples. After the plants were harvested, the solution in each culture jar was readjusted to the original 100-ml. volume, and six 1-ml. samples were counted to determine residual radioactivity. The difference between the initial and final counts was assumed to represent ^{14}C removed by the plants. Possible volatilization of ^{14}C-labeled compounds from solution, or losses of $^{14}CO_2$ from the leaves of plants, were not determined. Self-absorption corrections were made on the counts of all samples (*1*).

The dry, extracted plant material was ground in a Wiley mill. The ground plant meal was evenly distributed over a flat-bottomed planchet for counting. Self-absorption corrections followed the procedure used by Foy (*6*) and Sheets (*13*).

Radioactivity on thin-layer chromatograms was determined with a hand counter attached to a gas flow proportional system. Developed radioautograms were used to locate radioactive areas on chromatograms. Individual spots were isolated and counted. Counts of all radioactive areas developing from a single spotting were totaled and percentage values calculated for each area.

Results and Discussion

ED_{50} values for oat, soybean, corn, and cotton after 11 days' exposure to monuron and diuron in culture solution revealed that the order of susceptibility of species to both herbicides was: oat > soybean > corn > cotton (Table I). On a molar basis, diuron appeared to be more phytotoxic to all species than monuron. Statistically this could be shown only for cotton. Cotton was markedly more resistant than the other species to these herbicides.

Visible injury symptoms (chlorosis in primary leaves) were apparent in soybean at 48 hours, and injury progressed with time. Cotton showed no visible injury after 120 hours. Total ^{14}C uptake curves for cotton and soybean (actually the difference between the initial

Table I. Mean ED_{50} Values[a]

Crop	Monuron ($\times 10^{-7}M$)	Diuron ($\times 10^{-7}M$)
Oat	3.0	2.3
Soybean	4.9	3.2
Corn	10.5	7.8
Cotton	60.3	34.2

[a] Expressed as molar concentration required to reduce the fresh weight of seedlings 50%, 11 days after exposure to the herbicide

and final amount of ^{14}C in the solution culture) are presented in Figure 1. Since cotton removed more ^{14}C than soybean over the exposure time, differential absorption could not explain the wide difference in susceptibility of the two species. Divergence of the two uptake curves between 24 and 48 hours may be a reflection of the herbicidal effect of diuron on soybean.

Distribution patterns of alcohol-soluble ^{14}C in the plant parts are shown in Figure 2; the counts are

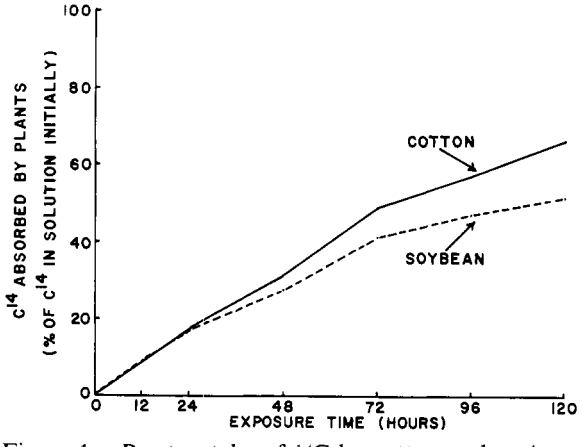

Figure 1. Root uptake of ^{14}C by cotton and soybean plants

Exposed to ^{14}C-diuron ($1.07 \times 10^{-6}M$) as a function of time

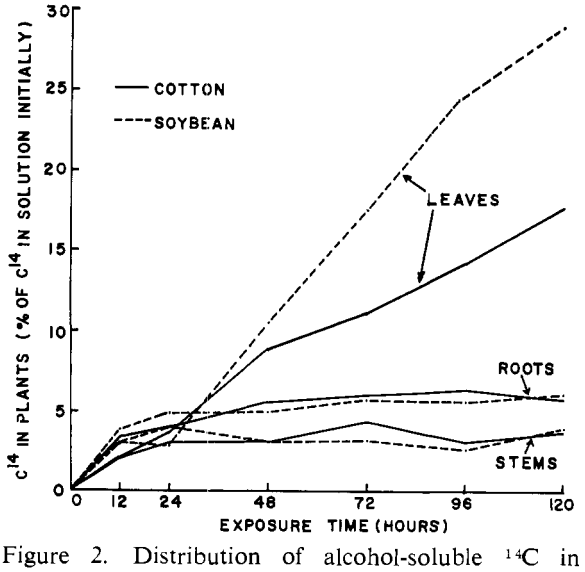

Figure 2. Distribution of alcohol-soluble ^{14}C in cotton and soybean plants

Roots exposed to ^{14}C-diuron ($1.07 \times 10^{-6}M$) as a function of time

expressed as per cent of ^{14}C present in the original solution. After an initial uptake during the first 12 hours (the shortest time interval used), the amount of ^{14}C in roots and stems of both plants remained relatively constant, with little or no additional accumulation. Alcohol-soluble ^{14}C accumulated in the leaves of both species but in larger quantities in soybean than in cotton at the long exposures. However, the cotyledons of soybean were extracted with the leaves, and it was later found that soybean exposed to ^{14}C-diuron accumulated sizable quantities of ^{14}C in the cotyledons.

The distribution of residual radioactivity in alcohol-extracted plant tops and roots is given in Figure 3. Nonalcohol-soluble ^{14}C accumulated in the roots of cotton over the time course and at 120 hours amounted to 18% of the ^{14}C in the original culture solution. Nonalcohol-soluble ^{14}C was also found in the roots of soybean; the level reached a peak at 72 hours and declined thereafter. Aerial plant parts of both species contained only small quantities of the nonalcohol-soluble fraction. In this experiment recovery of ^{14}C, after exposure of the plants, ranged from 80 to 105%. Lowest recoveries were found at the long exposure times with cotton. Although the ^{14}C distribution patterns were somewhat different in these two species, they offer no obvious explanation for the approximately 10-fold difference in the ED_{50} values.

After 120 hours' exposure of four species to ^{14}C-diuron ($1.07 \times 10^{-6}M$), removal of ^{14}C from the original treatment solution was determined for oat (23%), soybean (42%), corn (38%), and cotton (64%). Additional calculations revealed that the ^{14}C removed from solution per gram of fresh weight was 34% for oat, 15% for soybean, 6% for corn, and 16% for cotton. The amounts of ^{14}C in roots and tops of these species are shown in Figure 4. The most conspicuous species difference was found between the nonalcohol-soluble ^{14}C fraction of corn (1.6%) and that of cotton (18%). If the nonalcohol-soluble ^{14}C fraction represents altera-

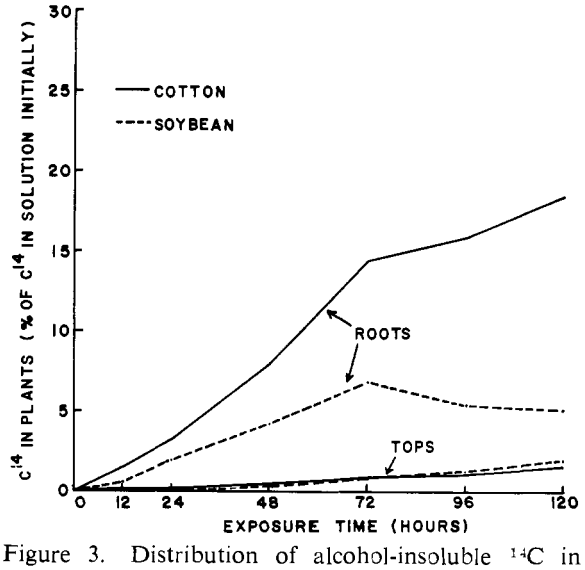

Figure 3. Distribution of alcohol-insoluble ^{14}C in cotton and soybean plants

Roots exposed to ^{14}C-diuron ($1.07 \times 10^{-6}M$) as a function of time

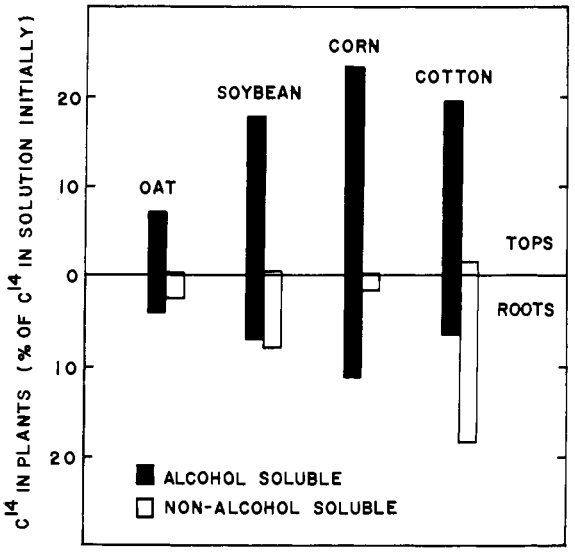

Figure 4. Distribution of alcohol-soluble and alcohol-insoluble ^{14}C in oat, soybean, corn, and cotton plants

Roots exposed to ^{14}C-diuron ($1.07 \times 10^{-6} M$) for 120 hours

tion of the parent herbicide, cotton appears to possess the greatest capacity to alter ^{14}C-diuron and corn the least.

In this study, soybean cotyledons were extracted separately from the remainder of the tops. It was revealed that of the 18% alcohol-soluble ^{14}C found in the tops of soybean, approximately one third accumulated in the cotyledons. The recovery of total ^{14}C after 120 hours' exposure was 91% for oat, 91% for soybean, 98% for corn, and 82% for cotton. After 120 hours of exposure, oat, soybean, and corn showed visible injury symptoms, but cotton did not.

Thin-layer chromatography was superior to paper for separation of the parent herbicides and their suspected intermediates. Hot alcohol extraction caused no alteration of ^{14}C-diuron.

Typical results from thin-layer chromatography of alcohol extracts from cotton and soybean exposed to ^{14}C-diuron for 120 hours are shown in Table II. The unidentified alcohol-soluble ^{14}C remained almost entirely at the origin. Since the phenylureas accumulate in the aerial portions of plants and are inhibitors of photosynthesis, composition of the leaf extracts was of major concern. Carbon-14–diuron was not identified in cotton leaves but 9% of the ^{14}C in soybean leaves remained as the unchanged herbicide. The major ^{14}C component (50%) in cotton leaves was identified as the nonphytotoxic 1-(3,4-dichlorophenyl)urea. The major ^{14}C component (48%) in soybean leaves was identified as the phytotoxic 1-(3,4-dichlorophenyl)-3-methylurea. The ^{14}C-diuron ($1.07 \times 10^{-6} M$) used in this study was below the ED_{50} for cotton but above that for soybean. This concentration proved favorable for comparing metabolism of diuron by a susceptible and a tolerant species. Higher concentrations of diuron might have resulted in detectable levels of diuron in cotton leaves. Chromatography of the nutrient solutions after the 120-hour exposure of soybean and cotton revealed that 62% of the ^{14}C in the soybean culture jar remained as ^{14}C-diuron. The corresponding solution for cotton showed only 20% as the unchanged herbicide and 61% as an unidentified fraction, which remained on the origin of thin-layer plates during chromatography. Small amounts of the monomethyl and demethylated derivatives were in both solutions.

Results of chromatographic analysis of alcohol extracts of cotton and soybean exposed to ^{14}C-monuron ($2.52 \times 10^{-6} M$) for 120 hours are presented in Table II. Cotton and soybean leaves contained 3 and 24%, respectively, of the ^{14}C as the unchanged herbicide. The major labeled component (58%) identified in cotton leaves was 1-(*p*-chlorophenyl)urea. A major labeled component (29%), identified as 1-(*p*-chlorophenyl)-3-methylurea, was found in soybean leaves. With the ring-labeled monuron, labeled, nonphytotoxic *p*-chloroaniline was identified in leaves of cotton (5%), but this derivative was absent from soybean. These values may have been reduced by volatilization of *p*-chloroaniline during solvent evaporation. The ^{14}C values for monuron and its derivatives in soybean leaves in Table II did not include ^{14}C found in the cotyledons.

Table II. Percentages of ^{14}C-Labeled Compounds in Various Plant Sections[a]

		Cotton			Soybean		
R_f[b]	Compound	Root	Stem	Leaf	Root	Stem	Leaf
0.63	Diuron	8	8	0	39	29	9
0.49	Monomethyl derivative	28	25	20	23	35	48
0.26	Demethylated derivative	17	40	50	5	13	25
0.00	Unknown	47	27	30	33	23	18
0.57	Monuron	12	20	3	27	40	24
0.43	Monomethyl derivative	12	26	13	12	20	29
0.21	Demethylated derivative	20	27	58	5	18	19
0.78	*p*-Chloroaniline	6	6	5	0	0	0
0.00	Unknown	50	21	21	56	22	28

[a] Determined by thin-layer chromatography of alcohol extracts from cotton and soybean plants after root absorption of ^{14}C-diuron ($1.07 \times 10^{-6} M$) and ^{14}C-monuron ($2.52 \times 10^{-6} M$) for 120 hours.
[b] Solvent system: benzene–acetone 2 to 1 (v./v.); chloroform–ethyl acetate 1 to 1 (v./v.) solvent system was employed for confirmation only and no data are reported.

Nutrient solutions, chromatographed after plants were grown therein, showed that 72% of the ^{14}C in the soybean culture jar was ^{14}C-monuron, whereas the corresponding solution for cotton contained 29% as the unchanged herbicide. The major ^{14}C fraction (42%) in the cotton culture solution was unidentified. The sources of labeled components in the nutrient solution, other than the parent herbicides and small quantities of monomethyl derivatives (impurities in the stock solutions), cannot be determined from these tests. Although microbial decomposition might be suspected, differences between plant species suggest that the labeled unknowns were more likely the result of plant metabolism. Additionally, it was not possible to demonstrate microbial decomposition in solution in the absence of plants. Chromatography of nutrient solutions, in which oat and corn were exposed for 120 hours, showed by visual estimates that the majority of the remaining ^{14}C was ^{14}C-diuron.

Results of chromatographic analysis of alcohol extracts from the tops of oat, soybean, corn, and cotton exposed to ^{14}C-diuron for 120 hours are given in Table III. These plants demonstrated varying abilities to alter the parent herbicide. The greatest change of diuron occurred in the tolerant cotton plant. Corn exhibited the least ability to alter the herbicide; in corn tops, 58% of the ^{14}C was ^{14}C-diuron. This result was surprising in view of the order of susceptibility of the four plants. However, results presented earlier (^{14}C removed per gram of fresh weight) indicated that, among the species studied, corn would have the lowest internal concentration of ^{14}C.

The cotyledons of soybean were extracted and chromatographed separately from the remainder of the tops. Some differences in amounts of the ^{14}C components were detected (Table III).

If we assume equal susceptibility among plants once the herbicide reaches the active site, the concentration of unchanged herbicide there determines the degree of plant injury. The concentration that can accumulate in the leaf is dependent on several plant processes: absorption; translocation, which is, in turn, dependent on transpiration; rate of increase in plant size; and rate and pathway of metabolic change of the herbicide. Except in corn, most of the ^{14}C which accumulated in the leaves of the four plants after root absorption was present as some compound other than the parent herbicide. The results suggest that the rate of metabolism is largely responsible for the tolerance of cotton to diuron. Differential metabolism appears to be a major factor contributing to the difference in susceptibility between cotton and the other three plants. Susceptibility differences observed among oat, soybean, and corn were best explained by differential absorption and growth rates (units of herbicide per units of fresh weight).

Table III. Percentages of ^{14}C-Labeled Compounds in Various Plant Sections[a]

Compound	Oat Tops	Soybean Tops	Soybean Cotyledons	Corn Tops	Cotton Tops
Diuron	12	9	5	58	5
Monomethyl derivative	40	54	71	26	11
Demethylated derivative	14	15	12	7	52
Unknown	34	22	12	9	32

[a] Determined by thin-layer chromatography of alcohol extracts from plants after root absorption of ^{14}C-diuron ($1.07 \times 10^{-6} M$) for 120 hours.

The site of herbicide breakdown in plants is not obvious from the results reported here. If we eliminate microbial decomposition and assume no downward translocation of the two intermediates, occurrence of the monomethyl and demethylated derivatives of these herbicides in plant roots and in the surrounding medium may indicate that part of the herbicide, at least, is metabolized in the roots.

The metabolites found in these studies strongly suggest the same breakdown pathway of the urea moiety of diuron in soil (*4*) and chloroxuron in plants and soil microorganisms (*7*). Under field conditions it would appear that plants, particularly tolerant plants, might contribute significantly to removal of phytotoxic residues from soils.

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