Distribution of Residues from Atrazine, Ametryne, and Pentachlorophenol in Sugarcane

H. W. Hilton, Q. H. Yuen, and N. S. Nomura

Atrazine, ametryne, and PCP, all labeled with ^{14}C, were applied to sugarcane and the movement of radioactivity in the plant was followed to determine the fate of their residues. Atrazine-^{14}C and PCP-^{14}C, applied to sugarcane leaf blades, remained at the site of treatment, but with substantial losses of radioactivity. As the leaves naturally abscised, all radioactivity was lost from the plant. PCP-^{14}C applied in nutrient solution to roots of growing plants was absorbed by the roots and did not translocate. With time, considerable radioactivity was lost from the roots and solution. Gas chromatography of the root extracts showed that less than 10% of the radioactivity was in the form of PCP. Atrazine-^{14}C and ametryne-^{14}C translocated readily from roots to green leaves with less than 10% of the total recoverable radioactivity in the stalk at any time; radioactive residues were lost from the growing plant as leaves abscised.

Gross chemical analysis for herbicides in sugarcane, which are applied only during the early growth stages, shows that residues of the parent compounds in the plant decrease with time; the decrease is attributed to dilution by growth, to weathering, to chemical and biological alteration, to volatilization, and to loss of the dry leaf trash. The crop period in Hawaii is between two and three years. In most cases, the parent herbicide residues decrease to undetectable quantities within eight months after application, but little is known of the fate of metabolic or chemical degradation products.

Only two previous attempts have been made to study the specific distribution of herbicide residues in sugarcane plant parts. It had been assumed that monuron and diuron, absorbed by roots from soil solution, were carried in the xylem to the leaves. Osgood (1969) confirmed this supposition using diuron-^{14}C. Hilton *et al.* (1968) applied diquat-^{14}C to sugarcane leaves and showed that the diquat and any possible metabolite disappeared from the growing plant within eight months by normal abscission of the treated leaves.

EXPERIMENTAL

Atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine], ametryne [2-(ethylamino)-4-(isopropylamino)-6-(methylthio)-*s*-triazine], and PCP (pentachlorophenol), labeled with ^{14}C in the triazine and benzene rings, respectively, were applied to sugarcane—atrazine and PCP to the leaves, and all three *via* nutrient solution to the roots. Quantities used for treatment were large enough to produce toxic symptoms: barely detectable stunting from atrazine, minor chlorosis and root toxicity from ametryne, moderate leaf and root injury from PCP.

Foliar Applications. Sugarcane of variety H 50-7209, growing in nutrient solution, was treated by painting both sides of the three top leaves with either atrazine-^{14}C or PCP-^{14}C. Atrazine (1.828 μCi. per mg., about 500 μg. per plant) was applied as an aqueous solution containing 10% methanol; PCP (0.043 μCi. per mg., 6000 μg. per plant) was applied as the sodium salt in water. Samples were taken from the atrazine-treated plants at 2 and at 8 weeks, and from the PCP-treated plants at 2, 4, and 8 weeks. The samples, consisting of treated leaves, untreated green leaves, dry leaf trash, stalk, roots, and suckers, were coarsely chopped and dried at 80° C. for 2 hours. Weighed portions were counted on 2-inch planchets in a Tracerlab G-M gas-flow counter; corrections were made from internal standards.

Experiment Station, Hawaiian Sugar Planters' Association, Honolulu, Hawaii 96822

Root Applications. Sugarcane plants of variety H 50-7209, growing in 3 liters of aerated nutrient solution in covered crocks, were treated by adding the herbicides, dissolved in methanol, to the nutrient solution. Concentrations of approximately 10 μg. per ml. for both atrazine and ametryne, and 5 μg. per ml. for PCP resulted from the following additions, in μg., to the nutrient solution:

Ring-labeled atrazine-^{14}C (1.828 μCi. per mg.)	312
Unlabeled atrazine	32,400
	32,712
Ring-labeled ametryne-^{14}C (5.58 μCi. per mg.)	328
Unlabeled ametryne	31,140
	31,468
PCP-^{14}C (0.043 μCi. per mg.)	15,000

The plants remained in the radioactive solution for 4 weeks; water and nutrients were added to maintain volume. After 4 weeks, the radioactivity remaining in the nutrient solutions had decreased 86% for PCP and about 70% for the two triazines. Aerated solutions without plants showed no significant loss of atrazine or ametryne, but extensive volatilization of PCP.

After the 4-week treatment period, the plants were removed, and the roots rinsed with water and placed in fresh nutrient solution without herbicides. Component parts of the triazine-treated plants—green leaves, dry leaf trash, stalk, roots, and suckers—were collected 1, 4, 8, and 13 weeks later. The same portions of PCP-treated plants were sampled at 0, 4, and 8 weeks. Samples were prepared and analyzed similarly to those from the foliar treatment. There was no leakage of radioactivity from the roots back into the fresh nutrient solution during the experiment.

RESULTS AND DISCUSSION

Foliar Application of Atrazine and PCP. Between the first and last sampling about 60% of the atrazine-^{14}C and 16% of the PCP-^{14}C had been lost from the plants. Since this radioactivity did not appear in any portions of the plants or in the nutrient solution in which the plants were growing, it was presumed lost from the treated leaves by vaporization or weathering, either as the parent compounds or as degradation fragments. The partially enclosed greenhouse where the experiments were conducted protected the plants from rain but not entirely from wind.

Table I. Residual Radioactivity after Foliar Application of Atrazine-^{14}C and PCP-^{14}C

Plant Part	Time after Application, Weeks	Dry Weight, G.	C.P.M./G.[a]	Total C.P.M.[a]	Distribution of Recoverable Radioactivity, %
		ATRAZINE-^{14}C			
Treated leaves	2	16.9	10,625	179,555	93
	8	17.7	4,575	80,980	96
Untreated green	2	23.6	280	6,605	3.4
leaves	8	52.4	30	1,570	1.9
Untreated dry					
leaf trash	8	19.5	70	1,365	1.6
Stalk	2	34.4	110	3,780	2.0
	8	109.3	0	0	0
Roots	2	14.1	200	2,820	1.5
	8	44.3	0	0	0
Suckers	8	1.3	110	145	0.08
Total plant	2	90.3	2,136	192,905	
	8	243.2	345	83,915	
		PCP-^{14}C			
Treated leaves	2	16.2	2,005	32,500	100
	4	11.0	2,970	32,170	82.1
	8	9.4	2,430	22,845	83.6
Untreated green	2	12.6	0	0	0
leaves	4	23.3	180	4,195	10.6
	8	43.3	40	1,805	6.6
Untreated dry	2	4.2	0	0	0
leaf trash	4	3.3	330	1,040	2.7
	8	9.3	60	545	2.0
Stalk	2	14.3	0	0	0
	4	19.4	95	1,845	4.6
	8	55.6	35	1,950	7.1
Roots	2	16.5	0	0	0
	4	15.6	0	0	0
	8	26.9	0	0	0
Suckers	8	6.2	30	195	0.7
Total plant	2	62.8	518	32,500	
	4	72.6	548	39,800	
	8	150.7	181	27,340	

[a] Corrected for self-absorption and background.

During the experiment, radioactivity which remained from both compounds was confined almost entirely to the treated leaves (Table I). Radioactivity from the PCP had been distributed to all parts of the plant except the roots, but in very small amounts; at eight weeks the counts were not significantly above background levels. Possibly traces of PCP vapor may have adhered to the untreated portions. Atrazine also left no significant amount of radioactivity at eight weeks in plant parts other than the treated leaves. The small amounts found in various plant parts at two weeks may be attributed to translocation of vapor or mechanical transfer to untreated portions. At eight weeks, the treated leaves were the lowest green leaves on the plants. As these leaves abscised—about one leaf every 10 days—the radioactivity was lost from the plant.

Application in Nutrient Culture Solution. PCP. Radioactivity decreased rapidly and exponentially in the nutrient solution. Of the 15.0 mg. of PCP-^{14}C applied, approximately 13.5 mg. were recoverable from the plants at the end of the four-week exposure period. During the subsequent eight weeks in fresh nutrient solution, radioactivity in the plants decreased 59%—indicating a PCP content of 5.5 mg. The loss took place from the roots without corresponding increases in radioactivity in the upper portions of the plants or in the nutrient solution (Table II).

The root system absorbed and retained over 99% of the total PCP taken up from solution. The remaining activity appeared in the stalk and suckers. Electron-capture gas chromatography of root extracts esterified with diazomethane indicated that less than 10% of the residue had a retention time identical to that of PCP. Small amounts of the residue were identical with tetrachlorophenol and trichlorophenol—these compounds appeared only as trace quantities in the original PCP. The remainder of the chromatographed extract exhibited two small peaks with longer retention times than any of the known chlorinated phenols. The loss of radioactivity from the roots may have been the result of either vaporization of volatile compounds released from the roots, or decomposition of root cells killed by the phytotoxic compound. However, the small amount of precipitate which could be isolated from the solution did not contain significant radioactivity.

Atrazine and Ametryne. In contrast to PCP, radioactivity in the nutrient solution with the two triazines decreased more slowly, dropping to 30% in four weeks. Radioactivity equivalent to about 180 μg. of atrazine (55%) and 160 μg. of ametryne (50%) could be detected in the plants soon after replacement in fresh nutrient solution. Decreases in total radioactivity recoverable from the plants took place over the 13-week period in fresh nutrient solution—49% of the atrazine and 38% of the ametryne were lost (Table II). Radioactivity from atrazine was lost mainly from the leaves; ametryne radioactivity was lost both in the root system and in the leaves.

In contrast to PCP, translocation of radioactivity to the leaves occurred readily with the triazines; only small amounts

Table II. Uptake and Distribution of Radioactivity from PCP-^{14}C, Atrazine-^{14}C, and Ametryne-^{14}C in Nutrient Solution

Plant Part	Time after ^{14}C Removal, Weeks	Dry Weight, G.	C.P.M./G.[a]	Total C.P.M.[a]	Distribution of Recoverable Radioactivity, %
		ATRAZINE-^{14}C			
Dry leaf trash	1	2.0	8,100	16,195	13.8
	4	11.8	3,545	41,835	47.3
	8	16.6	2,510	41,630	60.6
	13	29.8	1,410	41,945	69.4
Green leaves	1	20.8	3,830	79,690	67.7
	4	31.4	845	26,600	30.0
	8	44.8	95	4,350	6.3
	13	51.0	9	455	0.8
Stalk	1	6.6	520	3,420	2.9
	4	19.7	175	3,430	3.9
	8	40.8	115	4,795	7.0
	13	65.8	25	1,565	2.6
Roots	1	6.0	3,060	18,355	15.6
	4	12.9	1,290	16,625	18.8
	8	19.6	880	17,215	25.0
	13	51.8	310	16,135	26.7
Suckers	8	8.8	90	770	1.1
	13	6.8	45	320	0.5
Total plant	1	35.4	3,324	117,660	
	4	75.8	1,167	88,490	
	8	130.6	526	68,760	
	13	205.2	294	60,420	
		PCP-^{14}C			
Dry leaves (trash)	0	5.7	0	0	0
	4	4.3	0	0	0
	8	10.0	0	0	0
Green leaves	0	17.4	0	0	0
	4	32.8	0	0	0
	8	29.7	0	0	0
Stalk	0	8.2	105	865	0.8
	4	22.0	130	2,830	3.1
	8	23.2	80	1,885	4.6
Roots	0	6.3	15,955	100,525	99.2
	4	17.3	5,155	89,170	96.9
	8	16.8	2,300	38,630	93.8
Suckers	8	12.4	55	660	1.6
Total plant	0	37.6	2,697	101,390	
	4	76.4	1,204	92,000	
	8	92.1	447	41,175	
		AMETRYNE-^{14}C			
Dry leaf trash	1	3.0	6,745	20,230	7.2
	4	5.4	10,645	57,485	21.9
	8	18.3	7,635	139,745	53.1
	13	25.3	4,175	105,620	61.3
Green leaves	1	17.0	7,750	131,740	47.1
	4	29.2	4,020	117,415	44.6
	8	42.6	405	17,165	6.5
	13	41.9	100	4,090	2.4
Stalk	1	6.8	2,905	19,745	7.1
	4	15.5	1,025	15,870	6.0
	8	42.8	350	15,095	5.7
	13	41.4	310	12,920	7.5
Roots	1	5.8	18,635	108,080	38.6
	4	10.2	7,100	72,435	27.5
	8	18.2	4,925	89,610	34.1
	13	24.5	1,990	48,705	28.2
Suckers	8	9.8	195	1,425	0.6
	13	12.8	75	990	0.6
Total plant	1	32.6	8,584	279,845	
	4	60.3	4,365	263,205	
	8	131.7	1,997	263,040	
	13	145.9	1,181	172,325	

[a] Corrected for self-absorption and background.

were retained in the stalk. Each newly emerging leaf contained less radioactivity than the one before it, the older leaves received and retained the major portion and this was lost from the plant as the leaves naturally abscised. The stalk thus did not contain a substantial amount of residue at any time (Table II).

Initially, there was less root retention of atrazine than of the more toxic ametryne. However, with time, the percentage of radioactivity in the root system was about equal for the two herbicides. Retention of ametryne in the roots appears to delay the translocation into the leaves; yet, as with atrazine, the major quantity of residue is eventually lost when the leaves abscise. Ametryne is more toxic to sugarcane than atrazine; this may partially explain the retention of ametryne by the roots if it is assumed that the more toxic compound impairs the ability of the roots to translocate substances to other parts of the plant.

Ultraviolet absorption spectrophotometry and chromatography of the nutrient solutions containing the triazines showed a rapid degradation of the parent herbicides to more polar compounds. The so-called hydroxyatrazine [2-(ethylamino)-4-(hydroxy)-6-(isopropylamino)-*s*-triazine] that would be formed by hydrolysis of either atrazine or ametryne was not present. Unpublished data on chromatography of root and leaf extracts show that the parent herbicide and the hydroxyl analog are both absent.

The presence of the growing root system in the treated nutrient solution is necessary for the alteration of the compounds. No known model compound corresponds to the major radioactive constituent of the dry leaf trash—a polar, methanol–water 1:1 soluble substance with an absorption maximum of 244.8 mμ. Any consideration of chemical and/or biological degradation must account for the loss of a considerable proportion of the radioactive carbon. Attempts to trap $^{14}CO_2$ given off from the leaves failed to show any radioactivity in the barium carbonate precipitate.

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LITERATURE CITED

Hilton, H. W., Ospenson, J. N., Tucker, B. V., Yuen, Q. H., J. AGR. FOOD CHEM. **16,** 906 (1968).

Osgood, R. V., "Differential Tolerance of Hawaiian Sugarcane Varieties to Diuron, 3-(3,4-dichlorophenyl)-1,1-dimethylurea," Ph.D. dissertation, University of Hawaii, Honolulu, Hawaii, 1969.

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