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Radioisotopic and Gas Chromatographic Methods for Measuring Absorption and Translocation of 2,4,5-T by Mesquite[1]

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Abstract. The uptake and transport of carboxyl-labeled and unlabeled butoxyethyl esters and ammonium salts of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) by honey mesquite (*Prosopis juliflora* (Swartz) DC., var. *glandulosa* (Torr.) Cockerell) leaves were compared as measured by liquid scintillation counting and gas chromatography. Absorption of 2,4,5-T was determined by assaying leaf-rinsing solutions and extracts of treated leaves. Transport of 2,4,5-T was measured by determining amounts of 2,4,5-T found in stem tissue. The methods of analysis gave comparable results when extraction, cleanup, and analytical procedures were identical. When cleanup and methylation procedures were eliminated for the radioisotopic analyses, radioisotopic analyses gave significantly higher values for extracts of treated leaves than did gas chromatographic analyses. Larger amounts of 2,4,5-T were recovered from leaves treated with the butoxyethyl esters of 2,4,5-T than with ammonium salts.

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INTRODUCTION

RADIOISOTOPES and gas chromatography are used extensively to measure herbicides in plants and soils (1, 2, 3, 6, 7, 8); however, the literature contains little comparative information on the results obtained with these two methods. This paper describes experiments in which the two methods were used to measure the absorption and translocation of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) by honey mesquite (*Prosopis juliflora* (Swartz) DC., var. *glandulosa* (Torr.) Cockerell).

MATERIALS AND METHODS

Herbicides and treatments. High purity butoxyethyl esters of 2,4,5-T were obtained from commercial sources. The ammonium salt was prepared by reacting 2,4,5-T with ammonium hydroxide and adjusting the pH to 6. For radioisotopic analyses, carboxyl-labeled 2,4,5-T was diluted with unlabeled 2,4,5-T and water to an activity of 0.14 μc/50 μl and to a concentration of 1 μg of 2,4,5-T μl. All treatments contained 1.0% (v/v) polyoxyethylene tridecylether surfactant.

Plant materials. Three-year-old mesquite plants were

used in all experiments. The plants were grown in an irrigated nursery and were approximately 8 ft tall. In all instances, we pipetted 50 µg of herbicide on each leaf. Five adjacent leaves on a single stem constituted a treatment unit and four 5-leaf replications were used.

Extraction and analysis. Unabsorbed butoxyethyl esters of 2,4,=5-T were washed from treated leaves with two 50-ml portions of hexane and two 50-ml portions of 0.02 N ammonium hydroxide. The ammonium hydroxide and hexane washes were combined in a separatory funnel, shaken, and separated. An aliquot of the ammonium hydroxide fraction was evaporated and the 2,4,5-T esterified with 7 ml of BF_3-methanol solution (6). The esterified 2,4,5-T was taken up in 10 ml of hexane.

For radioisotopic analyses, the hexane portion containing methyl or butoxyethyl esters of 2,4,5-T was mixed with a scintillation solution which contained 2,4-diphenyloxazole (hereinafter referred to as PPO) and 1,4-bis (2-phenyloxazolyl) (hereinafter referred to as POPOP) at concentrations of 5 and 0.3 g/L of toluene, respectively. We used a Packard Tri-carb liquid scintillation counting system, Model 314EX[4], to assay samples for radioactivity. The herbicide contents of samples were determined by comparing the counts per minute of experimental samples to counts per minute from samples of known concentrations.

For gas chromatographic analyses, 1 µl of hexane solution was injected into a Barber-Coleman Model 5300 chromatograph[4] equipped with an electron-capture detector. Ra^{226} was the source of ionization. The injector, column, and detector temperatures were 290, 200, and 250 C, respectively. A 6-ft spiral, glass column, packed with 80-100 mesh Chromosorb W coated with 2% SE30 was employed. Flow rate of nitrogen carrier gas was approximately 75 ml/min for the methylated samples and 100 ml/min for the butoxyethyl ester samples. The herbicide contents of samples were determined by comparing peak heights of samples to the peak heights produced from samples of known concentrations.

Leaves treated with ammonium salts of 2,4,5-T were washed with four 50-ml portions of 0.02 N ammonium hydroxide to remove unabsorbed herbicide and processed in a manner identical to the ammonium hydroxide fraction described above.

To measure translocation of 2,4,5-T, stems supporting the treated leaves were harvested, placed in plastic bags, and stored at – 5 C until processed.

We cut leaf and stem tissues into 5-mm lengths and blended them twice with 50 ml of acidified acetone (Waring Blendor) for 4 min. The plant residue then was suction-filtered through Whatman No. 1 filtered paper. An aliquot of the acetone was evaporated and 2,4,5-T esterified with 7 ml of BF_3-methanol solution (6). The esterified 2,4,5-T was taken up in hexane and assayed for 2,4,5-T content by the methods described above for leaf washes.

Absorption and translocation of salts and esters. Leaves of 3-year-old mesquite plants were treated with ammonium salts and butoxyethyl esters of 2,4,5-T. Absorption and transport of 2,4,5-T were determined by harvesting treated leaves and stems supporting the treated leaves 0, 1, 6, and 24 hr after treatment and analyzing for their herbicide contents.

Comparison with cleanup and methylation eliminated for radioisotopic analysis. To further compare the two methods, leaves of mesquite plants were treated with ammonium salts and butoxyethyl esters of 2,4,5-T; treated leaves and stems supporting the treated leaves were harvested 0 and 24 hr after treatment and analyzed for 2,4,5-T content. The procedure for the radioisotopic analysis was altered slightly. Aliquots of the leaf wash and acetone extracts of the tissues were evaporated and taken up in the scintillation solution. Thus, the methylation and cleanup steps were eliminated.

Absorption and translocation by excised tissue. An experiment was initiated to determine if the loss of 2,4,5-T was due to translocation of 2,4,5-T out of the treated leaves into adjacent, untreated stem tissue. We cut 32 mesquite stems under water, kept the cut surface immersed in water, treated five leaves on each stem with 50 µg of the ammonium salt of 2,4,5-T, and harvested leaves and stems 0, 1, 6, and 24 hr after treatment. Water in which the cut stems were immersed during the treatment period was analyzed for 2,4,5-T content.

Results and Discussion

Qualitative study of esters. When we processed carboxyl-labeled standard of butoxyethyl ester of 2,4,5-T through the leaf-washing procedure, approximately 91 and 9% of the radioactivity appeared in the hexane and ammonium hydroxide fractions, respectively. By gas chromatographic analysis, the hexane and ammonium hydroxide fractions contained 89 and 11% of the 2,4,5-T. We concluded that the ester formulations of 2,4,5-T contained about 10% free acid. When mesquite leaves were included in the processing, we found approximately 81, 12, and 7% of the radioactivity in the hexane, ammonium hydroxide, and treated leaves, respectively. By gas chromatographic analysis, about 83, 10, and 7% of the 2,4,5-T occurred in the above fractions. Although the leaves were washed immediately after treatment, we were unable to remove all of the butoxyethyl ester of 2,4,5-T from the mesquite leaves. Both methods detected from 3 to 10% of the 2,4,5-T in the extracts of treated leaves.

Two peaks, which we designated "F" and "W", were recorded when the butoxyethyl ester of 2,4,5-T was injected into the gas chromatograph (Figure 1). Since the butoxyethyl ester of 2,4,5-T standard contained both n-butoxy and iso-butoxy ethyl esters of 2,4,5-T[5], we calculated the quantities of butoxyethyl ester of 2,4,5-T in experimental samples by comparing peak heights of samples with the two peaks produced by standard samples.

Table 1 contains data from an experiment conducted to determine if the two esters were absorbed at the same rate by leaves of mesquite. Both compounds disappeared from the hexane leaf washing solutions at approximately the same rate. The amounts of 2,4,5-T reported in the hexane washes are the sum of the two compounds.

[4]Use of trade names is for purposes of identification of equipment employed and does not constitute endorsement by the U. S. Department of Agriculture or Texas A&M University.

[5]J. Russell Bishop. 1967. Personal communication, Amchem Products Inc.

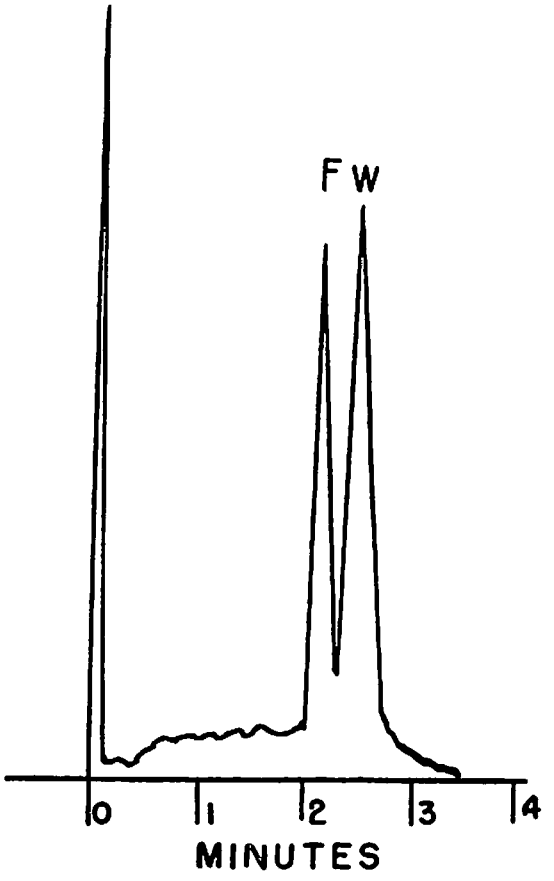

Figure 1. Gas chromatogram of butoxyethyl esters of 2,4,5-T in hexane leaf wash showing retention times of F and W peaks.

Table 1. Micrograms of butoxyethyl esters of 2,4,5-T recovered at four time-intervals in hexane wash after application of 250 μg to mesquite leaves.

Time after treatment hr	F peak	W peak	Total
0	100.6	101.8	202.4
1	87.0	85.3	172.3
6	40.3	40.8	81.1
24	14.2	20.4	34.6

Absorption and translocation of salts and esters. Amounts of esters of 2,4,5-T found in the hexane leaf wash decreased with time after treatment (Table 2). However, 2,4,5-T in the ammonium hydroxide leaf wash increased 1 hr after treatment. The rapid hydrolysis of esters of 2,4-dichlorophenoxyacetic acid (2,4-D) by leaves of grasses has been documented by Crafts (1) and Klingman *et al.* (5), and apparently mesquite leaves are capable of hydrolyzing the esters of 2,4,5-T. Since the 2,4,5-T was in the leaf wash, hydrolysis apparently occurred on the surface of the leaf or the converted form was washed from within the leaf during leaf-washing.

The amounts of 2,4,5-T increased in the treated leaves with time. The amounts of 2,4,5-T found in treated leaves as determined by the two methods did not differ significantly; however, values obtained by radioisotopic analysis usually were slightly higher than were values obtained by gas chromatographic analysis.

Stems supporting the treated leaves contained relatively small quantities of 2,4,5-T.

The ammonium hydroxide leaf wash removed about 98% of the ammonium salts of 2,4,5-T from the treated leaves when washing was done immediately after treatment. As time after treatment increased, we recovered smaller quantities of 2,4,5-T in the leaf wash and larger quantities in the treated leaves. However, the increases in the treated leaves were not as great as the decreases in the leaf wash. Consequently, the percentage of recovery decreased. Recovery from salt treatments were substantially lower than those from ester treatments.

Comparison with cleanup and methylation eliminated for radioisotopic analysis. Again, we were unable to wash all of the esters from the treated leaves immediately after treatment (Table 3). Smaller amounts of ester of 2,4,5-T

Table 3. Micrograms of 2,4,5-T recovered 0 and 24 hr after application of 250 μg of ammonium salts and butoxyethyl esters to mesquite leaves.

Material analyzed	Radioisotopic analysis[a]		Gas chromatographic analysis	
	0 hr	24 hr	0 hr	24 hr
	Butoxyethyl esters of 2,4,5-T			
Hexane leaf wash	207.0	28.4	190.2	63.5
Ammonium hydroxide leaf wash	30.2	0.4	45.0	17.8
Treated leaf extract	12.8	157.5	14.8	107.0
Stem supporting treated leaves	0.0	0.3	0.0	1.1
Total recovered	250.0	186.6	250.0	189.4
	Ammonium salts of 2,4,5-T			
Ammonium hydroxide leaf wash	249.7	18.0	243.7	16.0
Treated leaf extract	0.3	120.0	6.3	46.0
Stem supporting treated leaves	0.0	0.3	0.0	0.9
Total recovered	250.0	138.3	250.0	62.9

[a]Cleanup and methylation procedures eliminated for radioisotopic analysis.

Table 2. Micrograms of 2,4,5-T recovered 0, 1, 6, and 24 hr after application of 250 μg of ammonium salts and butoxyethyl esters of 2,4,5-T to mesquite leaves.

Material analyzed	Radioisotopic analysis				Gas chromatographic analysis			
	0 hr	1 hr	6 hr	24 hr	0 hr	1 hr	6 hr	24 hr
	Butoxyethyl esters							
Hexane leaf wash	201.8	119.3	44.3	12.5	202.4	162.3	81.1	34.6
Ammonium hydroxide leaf wash	29.7	47.8	19.5	31.7	21.8	37.9	17.7	13.8
Extract of treated leaves	18.5	55.3	124.9	150.2	25.8	49.6	115.2	112.7
Stem supporting treated leaves	0.0	0.1	0.6	0.2	0.0	0.2	0.9	1.4
Total recovered	250.0	222.5	189.3	194.6	250.0	250.0	214.9	162.5
	Ammonium salts							
Ammonium hydroxide leaf wash	249.0	185.9	93.1	21.0	245.8	194.3	107.6	27.0
Extract of treated leaves	1.0	16.0	43.2	75.2	4.6	13.2	65.2	61.5
Stem supporting treated leaves	0.0	0.2	0.1	0.2	0.0	0.4	1.1	2.0
Total recovered	250.0	202.1	136.4	96.4	250.4	207.9	173.9	90.5

were found in leaf washes by radioisotopic analysis than by gas chromatographic analysis. However, considerably higher levels of 2,4,5-T were found in the extracts of treated leaves by radioisotopic analysis. Partial metabolism of the 2,4,5-T molecule resulting in its elimination from the extract during the cleanup procedure could account for this difference.

Both methods of analysis show more 2,4,5-T recovered from plants treated with the esters than from plants treated with the salts. This is in agreement with results of the first experiment. Hull (4) has shown that salts of 2,4,5-T are more mobile than esters in greenhouse-grown mesquite plants. These data indicate that esters of 2,4,5-T are more strongly retained in leaves of field grown mesquite than are salts.

Absorption and translocation by excised tissue. Quantities of 2,4,5-T found in the leaf washes, treated leaves, and stems were approximately the same when determined by the two methods of analysis (Table 4). Slightly higher values were obtained for the leaf washes with gas chromatography than with radioisotopes, and slightly higher values were obtained from the extracts of treated leaves with radioisotopes than with gas chromatography. With increasing time after treatment, less 2,4,5-T was found in the leaf washes, with the largest loss occurring between 0 and 1 hr. Content of 2,4,5-T in the treated leaves increased with time after treatment. The amounts of 2,4,5-T found in the stems supporting the treated leaves always were less than 1.0 μg. Amounts of 2,4,5-T in the steep water also were small.

Total amounts of 2,4,5-T recovered from treated and untreated tissues were comparable to amounts recovered when intact stems were used at the 0 and 1-hr harvests, but 6 and 24 hr after treatment the recovery percentage did not decrease sharply as with intact stems (Table 2). Thus, it is evident that absorption and translocation data obtained with excised tissues are not always comparable to data obtained with intact plants. Largest quantities of 2,4,5-T were recovered from treated leaves. Since the amounts of 2,4,5-T found in the stems did not change when cut stems were used, changes in recovery were not due to changes in translocation patterns. Possibly the metabolism of the 2,4,5-T molecule or other factors such as mechanical loss of 2,4,5-T from the leaf surface were responsible for differences in recovery of 2,4,5-T observed in this study.

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Table 4. Micrograms of 2,4,5-T recovered 0, 1, 6, and 24 hr after application of 250 μg of ammonium salts to mesquite leaves.

Material analyzed	Radioisotopic analysis				Gas chromatographic analysis			
	0 hr	1 hr	6 hr	24 hr	0 hr	1 hr	6 hr	24 hr
Ammonium Hydroxide leaf wash	249.3	159.9	97.9	62.8	250.0	169.2	98.2	75.7
Treated leaf	0.7	39.0	104.8	162.5	T*	37.0	84.0	126.9
Stem supporting treated leaves	0.0	0.6	0.2	0.6	0.0	T	T	T
Steep water	0.0	0.1	0.1	0.1	0.0	1.5	0.7	0.5
Total recovered	250.0	199.6	203.0	226.0	250.0	207.7	182.9	203.1

*T = <0.1 μg.