

Ethyl and Methyl Parathion Residues in Green and Cured Alfalfa

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Alfalfa plots in the three climatic areas of Ohio were treated with ethyl and methyl parathion at rates of $\frac{1}{2}$ and 1 pound of insecticide per acre. Samples of alfalfa from all the plots were analyzed by gas chromatography with thermionic detection for parathion residues periodically during and at the end of the 15-day no-harvest interval. The rate of disappearance of parathion residues was rapid during the first 3 to 5 days following application of insecticide but more gradual during the remainder of the

period. At the end of the 15-day period methyl parathion residues were at or below the 1 p.p.m. tolerance level on all plots except in one dry hay sample from a plot treated at 1 pound per acre. However, residues of ethyl parathion approached or were at the 1 p.p.m. tolerance level after 15 days in green alfalfa at the $\frac{1}{2}$ pound per acre application rate and exceeded the tolerance in all other analyzed samples.

Considerable ambiguity has existed in the past related to the analytical results of parathion residues on alfalfa following the use of the insecticide for alfalfa weevil control. The development of the thermionic detector for gas chromatographic analysis (Giuffrida, 1964; Karmen, 1964; Giuffrida *et al.*, 1966; Ives and Giuffrida, 1967; Ford and Beroza, 1967) and the flame photometric detector (Brody and Chaney, 1966) have largely replaced the Averell-Norris (1948) colorimetric method for the determination of parathion residues in agricultural products (El-Refai and Hopkins, 1966; Nelson, 1967; Watts and Storherr, 1967; Wessel, 1967, 1968; Stevens, 1967).

Recent reports from western states (Stitt and Payne, 1966, 1967) indicated that excessive ethyl parathion residues were found on the alfalfa crop at harvest following the recommended 15-day no-harvest interval. Such reports gave rise to the question of whether excessive residues are due to local environmental conditions or might be prevalent at all locations where parathion is recommended for the control of alfalfa weevil. If the occurrence is widespread, it is questioned whether a residual hazard might result from applying ethyl parathion to alfalfa. Because both ethyl and methyl parathion have been recommended for alfalfa weevil control in Ohio, it was considered important to determine the rate of disappearance and the status of parathion residues at the time of harvest (15 days after the last application) as influenced by the conditions of the three climatic areas of the state.

EXPERIMENTAL PROCEDURES

Treatment of Alfalfa Fields. Alfalfa plots in three different areas of Ohio ranging in size from 0.2 to 1 acre were sprayed with emulsifiable concentrate formulations of ethyl and methyl parathion at the rates of $\frac{1}{2}$ and 1 pound actual insecticide per acre. The 13-foot boom hydraulic sprayer mounted

on a jeep was calibrated to deliver $\frac{1}{2}$ pound of insecticide in 20 gallons of liquid per acre. Two applications of the same concentration of insecticide, with a few minutes interval between applications, were made over the 1 pound actual insecticide per acre plots.

In southern Ohio (Waverly, Pike County) the plots were sprayed April 17 and again on May 2, 1967. In central Ohio (Columbus, Franklin County) plots were sprayed on May 10, 1967. In northern Ohio (Wooster, Wayne County) plots were sprayed on May 16, 1967. Alfalfa samples were collected at the intervals indicated in Tables IV and V following the last application of insecticide at each area. In all cases, larger samples were collected on the last day and part of the sample was dried on cardboard under open sunlight to simulate cured hay. All samples were chopped in a Hobart Food Chopper upon collection and stored in the freezer until analyzed.

Temperature and precipitation records were kept during the sampling period for the three different areas (Table I) for the purpose of comparing the effects of environmental conditions on residue levels. Rainfall on the day of application of insecticide in southern and northern Ohio did not interfere with application and permitted sufficient time for the spray to dry on the plants.

Condition of the alfalfa at Waverly was notably different from that in Columbus and Wooster. In the latter two areas, the alfalfa grew well and experienced only light weevil damage. Weevil damage accompanied by poor growing conditions at Waverly resulted in a very poor stand of alfalfa with only spotty growth confined mainly to the higher ground areas. It became difficult to find random alfalfa samples throughout the plots. Weevil damage in unsprayed plots was so extensive that collection of control alfalfa became increasingly difficult as the study progressed.

ANALYTICAL METHODS

The dry weight of green alfalfa and alfalfa hay samples was calculated by drying the samples in an oven to a constant

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Table I. Temperature and Precipitation Records during Experiment, May 1967

Sampling Period, Days	Waverly, Ohio, May 2-17			Columbus, Ohio, May 10-25			Wooster, Ohio, May 16-31		
	Temp.		Pctn., In.	Temp.		Pctn., In.	Temp.		Pctn., In.
	Max.	Min.		Max.	Min.		Max.	Min.	
0	76-51		0.34	63-36		...	58-39		0.05
1	57-31		0.20	71-50		1.2	64-38		...
2	61-38		...	54-44		...	73-43		0.10
3	69-41		...	65-42		...	73-60		T ^a
4	70-46		0.27	62-49		0.12	68-45		...
5	58-44		1.88	52-46		0.65	61-41		...
6	58-42		0.14	59-39		...	56-37		...
7	61-45		0.19	66-47		0.10	68-32		...
8	57-33		T	73-45		...	72-50		...
9	67-45		0.68	74-57		...	75-46		...
10	80-51		...	68-45		...	72-43		...
11	63-47		...	69-43		...	87-50		...
12	66-50		0.20	64-42		...	74-51		0.81
13	67-48		0.84	60-35		...	60-45		0.23
14	53-38		0.19	68-50		...	66-40		0.24
15	62-43		0.34	76-53		...	66-48		...
	Av. 64.1-43.3		Tot. 5.27	Av. 65.1-45.2		Tot. 2.07	Av. 68.3-44.2		Tot. 1.43

^a Trace.

weight. Alfalfa hay was 88% dry matter and green alfalfa 20% dry matter.

Fifty-gram portions of the same green alfalfa sample collected from the field 3 days after application of insecticide were extracted by 5 different procedures to determine the efficiency for extraction of both ethyl and methyl parathion. The methods were: (1) Soxhlet extraction for 16 hours with 300 ml. of 10% ethanol in benzene; (2) blending for 15 minutes, at relatively low speed regulated at 45 watts on the Variac, in a Waring Blendor with 300 ml. of 65% acetonitrile in water; (3) blending under the same conditions as above with 300 ml. of acetonitrile; (4) blending, same conditions, with 300 ml. of hexane; and (5) blending with 300 ml. of hexane following the addition of 100 grams of anhydrous sodium sulfate. Measurement of the parathion residues (Table II) showed very little differences in the extraction efficiency of procedures 1, 2, 3, and 4 and much less efficiency in procedure 5. Because of the greater ease in procedure, the hexane (No. 4) extraction of 50-gram green alfalfa samples was used throughout the study. The 15-minute blending time at 45 watts resulted in about 3-4° C. increase in temperature. Covered blending jars and filtration equipment were used to minimize evaporation losses.

With the exception of the hexane-sodium sulfate extraction method, the procedures cited above were repeated with 25-gram portions of the same alfalfa hay samples. In this case the Soxhlet extraction was much more efficient for the ex-

traction of ethyl parathion and the 65% acetonitrile and Soxhlet extractions were equal in efficiency for the extraction of methyl parathion. Consequently, Soxhlet extraction of 25 grams of hay was used for ethyl parathion samples and 65% acetonitrile extraction for methyl parathion samples because of the time factor involved.

The alfalfa residues from the samples cited previously were reextracted two additional times by the same procedures. Extraction of additional ethyl and methyl parathion was insignificant. The efficiency of the extraction methods used in this study was thus considered reliable and comparable to other recently reported procedures (Archer and Crosby, 1967, 1968; Root, 1967; Ware *et al.*, 1968; Whiting *et al.*, 1968).

The benzene was evaporated from an aliquot of the Soxhlet extract. The remaining aqueous alcoholic residue was mixed with 700 ml. of 2% Na₂SO₄ and partitioned 2 times with 100-ml. aliquots of hexane by vigorous shaking in a separatory funnel. One hundred ml. of the 65% acetonitrile extract was partitioned by the same procedure. All extracts, now in hexane, were cleaned by passing them through columns containing 25 to 30 grams of activated Florisil according to the procedure outlined by Stitt and Payne (1966). Ethyl and methyl parathion residues were eluted in the 1:1 benzene-diethyl ether solvent according to the procedure for hexane and Soxhlet extracts, but in the benzene eluate for the 65% acetonitrile extract. Consequently the benzene wash of the column containing the residue of the 65% acetonitrile extract was omitted, and the residues were eluted in the benzene-diethyl ether solvent.

An F & M Biomedical 400 Gas Chromatograph equipped initially with a sodium thermionic detector was used for detection and measurement of parathion residues. In later analyses, the sodium sulfate was replaced on the coil with rubidium sulfate and/or potassium iodide, which greatly increased the sensitivity, and finally with potassium chloride. Separation was accomplished on a 1/4-inch O.D., 4-ft. glass column containing 3.8% SE-30 on 80 to 100 mesh Diatapore S. The column had been in use for several months and had been conditioned by heating at 250° C. for 24 hours and then reheating periodically over the months to remove extraneous materials. The operating conditions for the chromatograph

Table II. Comparison of Methods in Extracting Methyl and Ethyl Parathion Residues from Alfalfa

Method of Extraction	Parathion Residue Extracted, P.P.M.			
	Green Alfalfa ^a		Alfalfa Hay ^a	
	Methyl	Ethyl	Methyl	Ethyl
1. Soxhlet	6.53	10.4	0.78	3.87
2. 65% Acetonitrile	6.39	10.1	0.77	2.80
3. Acetonitrile	7.92	9.39	0.63	1.87
4. Hexane	6.54	9.92	0.29	2.80
5. Hexane + anhy Na ₂ SO ₄	5.87	7.41	—	—

^a All subsamples for each column series were obtained, respectively, from the same chopped and mixed green alfalfa or dry alfalfa hay samples.

Table III. Recovery of Methyl and Ethyl Parathion from Alfalfa

Parathion, P.P.M.				Recovery % ^a	
Added		Found ^a		Methyl	Ethyl
Methyl	Ethyl	Methyl	Ethyl		
GREEN ALFALFA-HEXANE EXTRACTION					
0.00	0.00	0.00	0.00		
0.00	0.00	0.00	0.00		
0.00	0.00	0.01	0.01		
0.00	0.00	0.01	0.01		
0.00	0.00	0.00	0.00		
0.00	0.00	0.00	0.00		
		Av. 0.00	-0.00		
0.22	0.17	0.17	0.13	77.3	76.5
0.22	0.17	0.16	0.12	72.8	70.6
0.4	0.4	0.40	0.35	100.0	87.5
0.4	0.4	0.35	0.34	87.4	85.0
0.44	0.43	0.39	0.35	88.9	81.5
0.44	0.43	0.43	0.37	97.9	86.0
0.65	0.68	0.57	0.58	87.8	85.5
1.09	1.02	1.00	0.88	91.6	86.3
1.09	1.02	0.94	0.79	86.3	77.4
2.0	2.0	2.21	2.07	110.5	103.5
2.0	2.0	1.81	1.71	90.5	85.5
10.0	10.0	9.24	8.81	92.4	88.1
10.0	10.0	8.22	7.78	82.2	77.8
40.0	40.0	39.3	38.1	98.3	95.2
40.0	40.0	31.7	29.1	79.2	72.6
				Av. 89.5	83.9
ALFALFA HAY-65% ACETONITRILE EXTRACTION					
0.00	0.00	0.00	0.00		
0.00	0.00	0.00	0.00		
		Av. 0.00	0.00		
0.62	0.96	0.53	0.85	85.5	88.5
0.77	4.80	0.81	4.10	105.1	85.4
1.54	2.40	1.55	2.44	100.6	101.3
2.31	1.92	2.36	1.85	102.1	96.4
				Av. 98.3	92.9
ALFALFA HAY-SOXHLET EXTRACTION					
0.00	0.00	0.00	0.00		
0.00	0.00	0.00	0.04		
0.00	0.00	0.00	0.02		
		Av. 0.00	0.02		
0.31	0.96	0.27	0.88	87.0	91.6
0.46	0.48	0.35	0.47	76.1	97.9
0.77	4.80	0.67	4.41	87.0	92.0
0.77	4.80	0.74	3.91	96.1	81.5
2.31	1.92	1.88	1.70	81.4	88.5
				Av. 85.5	90.3

^a Recovery values corrected for apparent residue in the controls.

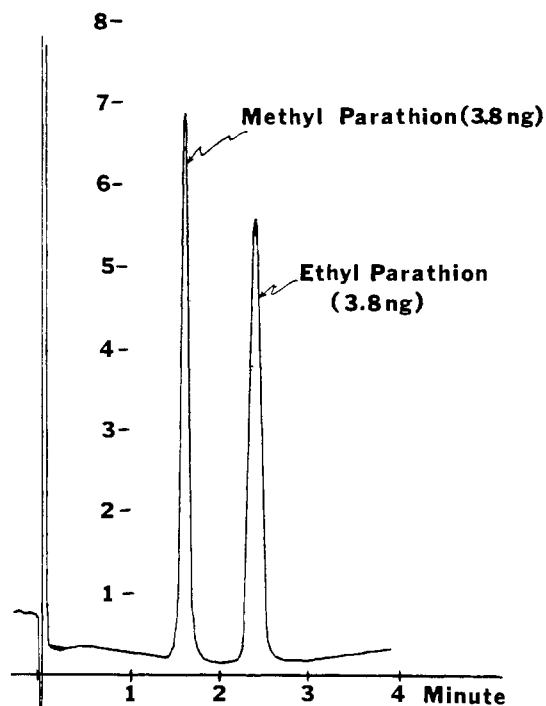


Figure 1. Gas chromatographic separation of methyl and ethyl parathion in alfalfa hay

Column, glass, 4 ft., 1/4 in. O.D., 3.8% SE-30 on 80 to 100 mesh Diataport S (Hewlett-Packard), isothermal at 175° C. injection block 210° C.; Detector, KCl thermionic, 260° C. on F & M 400 Biomedical G. C.; Electrometer, range 100, attenuation 64, chart span 1 mv.; Gas flow, 60 ml. He carrier/min., 25 ml. H/min., 600 ml. Air/min. Av. sample volume injected 2 to 4 µl.

are shown in Figure 1. Concentrations of ethyl and methyl parathion residues were calculated from standard curves obtained periodically throughout each day concurrently with the analysis of samples. Linearity was satisfactory between 15 and 70% recorder scale deflection at the attenuations used. The retention times for ethyl and methyl parathion are shown on Figure 1.

RESULTS AND DISCUSSION

The recovery of ethyl and methyl parathion from green alfalfa and alfalfa hay by the methods used in this study is reported in Table III. These methods gave satisfactory recoveries over a range of 0.2 to 40.0 p.p.m. and could likely be extended in both directions beyond that concentration

Table IV. Apparent Parathion Residues in Alfalfa from Untreated Control Plots

Day of Sampling after Application	Methyl Parathion, P.P.M.			Ethyl Parathion, P.P.M.		
	Pike County ^a	Franklin County	Wayne County	Pike County ^a	Franklin County ^a	Wayne County
0	0.09	0.06	0.04	0.10	0.00	0.07
3	0.00	0.00	0.05	0.00	0.00	0.09
5	...	0.11	0.00	...
6	0.00	0.00
7	0.05	0.06
10	0.00	...	0.01	0.00	...	0.02
11	...	0.02	0.00	...
15	0.02	0.04	0.02	0.05	0.00	0.00
Av.	0.02	0.05	0.03	0.03	0.00	0.05
Dry Hay						
15	0.02	0.00	0.00	0.00	0.00	0.01

^a Two applications in Pike County 15 days apart. Sampling commenced after second application.

Table V. Summary of Methyl and Ethyl Parathion Residues in Green Alfalfa and Alfalfa Hay

Day of Sampling after Application	Methyl Parathion, P.P.M. ^b						Ethyl Parathion, P.P.M. ^b					
	$\frac{1}{2}$ Lb. per Acre			1 Lb. per Acre			$\frac{1}{2}$ Lb. per Acre			1 Lb. per Acre		
	Pike County ^a	Franklin County	Wayne County	Pike County ^a	Franklin County	Wayne County	Pike County ^a	Franklin County	Wayne County	Pike County ^a	Franklin County	Wayne County
0	47.6	31.2	25.4	60.7	50.8	49.2	40.0	26.4	32.8	103.0	68.0	57.0
3	8.84	3.69	4.48	19.4	7.10	11.5	11.3	4.94	9.17	28.2	11.1	14.8
5	...	1.17	3.11	3.41	7.43	...
6	3.07	6.90	5.63	20.4
7	1.40	3.86	2.96	7.22
10	0.96	...	0.89	1.69	...	1.46	2.25	...	1.28	4.76	...	2.98
11	...	0.30	0.86	1.13	2.52	...
15	0.20	0.14	0.11	0.43	0.36	0.44	1.05	0.57	0.56	3.68	1.43	1.52
Dry Hay												
15	1.08	0.28	0.52	2.57	0.77	1.16	4.23	2.38	1.82	6.25	4.70	4.25

^a Two applications in Pike County 15 days apart. Sampling commenced following second application.

^b Residue values corrected for apparent residue in controls and efficiency of analytical method.

range based upon previous work reported (Stitt and Payne, 1968) and on the sensitivity of the thermionic detector used. There were no significant interferences encountered in residue measurement that responded to the thermionic detector (Table IV). In fact, analysis of the raw extracts prior to partitioning and column cleanup did not result in significant interferences in pesticide measurement but, after a period of time, did reduce the efficiency of the gas chromatographic column. An electron capture detector was also sensitive to parathion residue measurement, but was restricted in its use because of its response to extraneous materials in the sample extracts analyzed after the cleanup procedure.

The results reported in Table V show a rapid decline in both ethyl and methyl parathion residues on green alfalfa during the 15-day sampling period with the greatest rate of disappearance during the first three days. At the end of the 15-day sampling period, the residue of methyl parathion on green alfalfa was well below the tolerance levels of 1 p.p.m. on all plots from all three counties. Residues of ethyl parathion on green alfalfa at the $\frac{1}{2}$ pound per acre rate were found to be at or slightly below the tolerance level at the end of 15 days and in excess of the tolerance at the 1 pound per acre rate. On the dry alfalfa hay samples, methyl parathion residues approached or were below the tolerance level on all samples except the Pike County sample at the 1 pound per acre application rate. Ethyl parathion residues were in excess of the tolerances on all samples. Calculation of the residues on a dry weight basis gave good correlation between the green alfalfa and respective hay samples.

Parathion residues found in alfalfa samples collected from Pike County in southern Ohio generally exceeded that found in samples from the other two counties at similar sampling intervals. The difference in the number of parathion applications as well as differences in weather conditions and consequently in the growth of the alfalfa likely contributed to the differences in residue levels. The effect of environmental conditions on residue levels was further substantiated by the results of preliminary investigations conducted on plots in Franklin County that were sprayed on September 13, 1966. Although some rain fell during the period, days were generally cool and the growth of alfalfa during the period was minimal. Samples collected on the 1st and 15th days after application of insecticide showed methyl parathion residues to be reduced from 8.5 to 0.48 p.p.m. at the 1 pound application rate and from 2.52 to 0.11 p.p.m. at the $\frac{1}{2}$ pound rate. Ethyl parathion residues, however, were reduced only from 22.37 and 11.47 p.p.m. on the 1st day at the 1 pound and $\frac{1}{2}$ pound application rates, respectively, to 8.89 and 1.96 p.p.m. on the 15th day. A similar environmental effect on the growth

of alfalfa in Pike County during the 15-day interval of the first spray application could account for the higher initial residue levels found and, consequently, the higher residues found at the termination of the experiment. Samples were not analyzed prior to the second spray application.

The results of this study indicate that methyl parathion applied at the rates of $\frac{1}{2}$ pound and 1 pound per acre in Ohio does not result in excessive residues at harvest time 15 days after application. On the other hand, the application of ethyl parathion, particularly at the 1 pound per acre rate, resulted in residues in excess of the 1 p.p.m. tolerance at the 15-day harvest period. Weather and growing conditions appeared to affect the rate of disappearance of ethyl parathion. This study thus tends to support claims from other areas of the nation regarding excessive residues of ethyl parathion in alfalfa at harvest.

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