Determination of 2,4-Dichlorophenoxyacetic Acid in Citrus Fruit

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A method for recovering 2,4-D from citrus fruit is described. The 2,4-D was made volatile by esterification with diazomethane and was detected as chloride by a microcoulometric gas chromatograph. Analyses of oranges picked from trees sprayed with 20 p.p.m. of 2,4-D showed an average of 0.1 p.p.m. of 2,4-D residue 1 day after spraying and lemons dipped in wax emulsion containing 500 p.p.m. of 2,4-D showed an average of 1.1 p.p.m. of 2,4-D residue 2 days after treatment.

In citrus culture, 2,4-D (2,4-dichlorophenoxyacetic acid, salt or ester) is used as a plant growth regulator in reducing preharvest fruit drop and increasing fruit size (*6*, *7*). It also has a valuable application in postharvest treatment of lemons to increase their storage life (*8*). Although various forms of 2,4-D have been tried over the years, the isopropyl ester has become the form most widely used.

This report is concerned with the development of a method for determination of 2,4-D in citrus fruit and with the presentation of some residue data for field-sprayed oranges and for lemons dipped in 2,4-D in a manner comparable to packing house treatment.

Instrument

The instrument used for detecting 2,4-D was a microcoulometric gas chromatograph (Dohrmann, 1960 model) which detects halogens in volatile organic compounds when operated oxidatively (*1*). The recorder used was a standard −0.5 to 5.0 mv. recorder (Bristol) equipped with a mechanical cam integrator. The chromatographic column was a 6-foot aluminum tube, 1/4 inch in O.D., packed with GC-22 firebrick packing which had been acid-washed and coated with 20% Dow-Corning high vacuum silicone grease previously purified by precipitation with ethanol from an ethyl acetate solution.

The instrument was operated under the following conditions: block, 260° C.; column, 250° C.; furnace, 820° C.; attenuation 128 ohms; damping 6.8 megohms (position 3). Nitrogen was used as the carrier gas and extra oxygen for combustion was introduced through the port normally used for hydrogen in reductive determinations of sulfur. The gas flow settings were varied until optimum conditions were found for the recovery of chloride from 2,4-D.

Reagents

Diazomethane for esterification of the 2,4-D was prepared according to the directions of Hickinbottom (*2*), starting with methylurea to make the intermediate, nitrosomethylurea. The latter was stored in the refrigerator to retard decomposition. The highly toxic diazomethane was freshly prepared, in the fume hood, in ether solution from nitrosomethylurea whenever a set of samples was ready to be esterified. One gram of nitrosomethylurea and 6 ml. of 70% aqueous KOH per 100 ml. of diethyl ether were used.

The phosphate buffer contained 25 grams of Na_2HPO_4 and 10 grams of NaH_2PO_4 per liter.

Procedure

Extraction of 2,4-D from Fruit. The 2,4-D was extracted from the citrus fruit with acetone in a concentration sufficient to precipitate pectic materials (*5*). Samples of 100 to 250 grams of chopped fruit were ground in a Waring Blendor with 100 to 250 ml. of acetone, transferred to a 1-liter boiling flask, and refluxed for 10 minutes to kill the cells and to permit the 2,4-D to diffuse out. After suction filtration, the pulp was reground in fresh solvent. The two acetone extracts were combined and evaporated in a rotary vacuum evaporator.

Further steps in the recovery of the 2,4-D were adapted from the method of Marquardt and Luce (*3*). One milliliter of HCl and 100 ml. of water were added to the concentrated extract. The 2,4-D was extracted with three 50-ml. portions of diethyl ether. At this point, some samples were separated into acid and ester fractions by extracting the ether solution with two 25-ml. portions of phosphate buffer. The phosphate buffer, containing the acid fraction, was acidified with 2 ml. of HCl and extracted with two 10-ml. portions of carbon tetrachloride. The solvent was evaporated with suction and the 2,4-D acid residue was ready for esterification.

To the ether solution above, containing total 2,4-D, or only ester, 100 ml. of water and 1 ml. of 50% NaOH solution were added. The ether was evaporated in a rotary vacuum evaporator with suction and slight warming. Then the flask was heated on the steam cone for 5 to 10 minutes to complete hydrolysis of the ester. The solution was cooled and acidified with 2 ml. of HCl. The 2,4-D was partitioned successively into ether, phosphate buffer, and carbon tetrachloride as above.

Esterification of 2,4-D. The samples of 2,4-D acid to be esterified were contained in 50-ml. Erlenmeyer flasks. Five milliliters of ether solution containing 12 ml. of methanol per 100 ml. of diethyl ether were added to the flasks to improve esterification (*4*). Next, 5 ml. of ether and diazomethane were added to the flasks to produce the methyl ester of 2,4-D. Usually, the esterification was performed late in the day so that the excess diazomethane, the ether, and the methanol could evaporate from the flasks left overnight in the fume hood at about 25° C. Because of the volatility of the methyl ester of 2,4-D, it was desirable not to have the solvents evaporated for very long before dissolving the ester in xylene and making up to volume for analysis.

Detection of 2,4-D. Recovery of chloride from purified methyl ester of 2,4-D dissolved in xylene was found to range from as low as 65% to as high as 95% using the Dohrmann instrument. However, on a given day of operation the recoveries were less variable. On one day, the recoveries were 83, 80, 84, 86, and 75% while on another day they were 74, 69, 72, 69, and 71%. A standard solution containing $1 \times 10^{-3}M$ methyl ester was made up in xylene and used as a reference each day the instrument

was operated. One or two injections of standard ester solution were introduced into the instrument to bring it to equilibrium before determinations were recorded. At an attenuation of 128 ohms, 15 μl. of this solution (3.525 μg. of ester) provided a satisfactory reference and a reproducible peak area could be obtained with as little as 0.5 μg. of methyl ester. Calculations were made according to the equation of Coulson *et al.* (*1*).

Preliminary Experiments. A test of the esterification procedure was made on purified 2,4-D acid. Five-milliliter aliquots of $1 \times 10^{-3}M$ 2,4-D acid solution were evaporated to dryness under reduced pressure, but without boiling, and the residue was esterified with diazomethane. Recovery of chloride from the esterified samples was 64, 66, 69, 67, and 68% as compared with 69, 67, 69, and 68% recovery from a standard methyl ester solution. These results indicated that the esterification was essentially complete as far as the accuracy of detection was concerned.

Time required for hydrolysis of the isopropyl ester of 2,4-D was determined at pH 10, 11, and 12 for one or two temperatures. Ten-milliliter aliquots of the ester in acetone solution ($1 \times 10^{-3}M$) were mixed with 10-ml. aliquots of aqueous $0.1M$ sodium bicarbonate-carbonate or carbonate-hydroxide mixtures which produced the desired pH values. After hydrolysis for various lengths of time, the pH was again determined and found to be mostly within 0.1 pH unit of the starting pH. Some hydrolyses were carried out at room temperature (about 25° C.) and others at the refluxing temperature of the solutions (about 65° C.). The latter preparations were refluxed on the steam cone for the desired time, cooled immediately in a cold water bath, the pH determined, and then transferred to a separatory funnel together with 130 ml. of water. The nonhydrolyzed ester was removed by extraction with two 25-ml. portions of ether. The water solution, left after extraction of the ester, was acidified with 1 ml. of HCl and extracted again with two 25-ml. portions of ether to recover the hydrolyzed 2,4-D. The 2,4-D was then partitioned into phosphate buffer and carbon tetrachloride before esterification.

The hydrolyses at room temperature were carried out in glass-stoppered Erlenmeyer flasks. After the desired period of time, the separation of acid and ester was effected as above.

The results showed that at pH 12, the isopropyl ester of 2,4-D was hydrolyzed in about 5 minutes at 65° C. or in about 2 hours by standing at 25° C. At pH 11, the hydrolysis required about 1 hour at 65° C. or about 3 days by standing at room temperature.

At pH 10 and room temperature, the hydrolysis of the ester was complete in about 7 days. This was of interest because the packing house application of 2,4-D to lemons uses the ester in a water-wax emulsion of about pH 10.3.

In addition to the hydrolysis experiments with the purified ester, others were made with isopropyl ester in wax emulsion. The water-wax emulsion was prepared to contain 1% of wax (diluted from 8% Sunkist water-wax stock emulsion), 1% of sodium carbonate, and 500 p.p.m. of 2,4-D acid equivalent introduced as the isopropyl ester in an emulsifiable, commercial product. The emulsion tested had a pH of 10.3.

Analysis of the wax emulsion for total 2,4-D content was carried out as follows: Five-ml. aliquots of the wax emulsion were pipetted into 250-ml. glass-stoppered Erlenmeyer flasks. Fifty milliliters of water plus four drops of 50% NaOH were added to raise the pH above 12. The contents were refluxed for 10 minutes, cooled, and transferred to a separatory funnel, using about 50 ml. of water to complete the transfer. Two milliliters of HCl were added, the 2,4-D was extracted with two 50-ml. portions of diethyl ether, and the usual procedure was followed.

Analysis of the wax emulsion for the hydrolyzed portion of 2,4-D after different times was performed in the same way as for total 2,4-D, except that the hydrolysis was omitted. The nonhydrolyzed 2,4-D was eliminated by partitioning into phosphate buffer.

The recovery of total 2,4-D from the freshly prepared wax emulsion averaged 92% after allowance was made for the incomplete recovery of chloride by the instrument. Hydrolysis of the ester in the wax emulsion during periods of standing at room temperature was 18% after 1 day, 92% after 6 days, and 96% after 11 days. Although the ester hydrolyzed readily in the wax emulsion, the reaction was not as rapid as previously noted when in solution.

Evaporation of the isopropyl ester of 2,4-D from a glass surface was measured. A commercial product (Esteron 44, Dow) containing the ester was diluted with acetone to produce a solution containing 500 μg. of 2,4-D acid equivalent per ml. One-milliliter aliquots of this solution were pipetted onto watch glasses in duplicate on 5 consecutive days. On the fifth day, after the acetone had evaporated from the last pipetting, the contents of the watch glasses were dissolved in xylene and analyses of the ester were made in the Dohrmann instrument. The ester was spread out in an area about 2 inches in diameter during exposure. Under these conditions, the ester was about 50% evaporated in 1 day, about 85% evaporated in 2 days, and essentially fully evaporated in 3 and 4 days.

Table I. Amount of 2,4-D Rinsed from Individual Washington Navel Oranges with Acetone while the Fruit Was Still Wet with a Spray of 20 P.P.M. of 2,4-D Acid Equivalent as the Isopropyl Ester or Rinsed 7 Days after Spraying

Fruit Weight, Grams	2,4-D Acid Equivalent Recovered, μg. Apparent	μg. Corrected[a]	Fruit Weight, P.P.M. Apparent	P.P.M. Corrected[a]
On Day of Spraying with 2,4-D				
147	28	15	0.2	0.1
175	37	24	0.2	0.1
223	32	19	0.1	0.1
187	34	21	0.2	0.1
217	38	25	0.2	0.1
122	29	16	0.2	0.1
205	36	23	0.2	0.1
197	37	24	0.2	0.1
164	30	17	0.2	0.1
162	22	9	0.1	0.1
171	38	25	0.2	0.1
153	32	19	0.2	0.1
172	23	10	0.1	0.1
154	30	17	0.2	0.1
Av. 175	32	19	0.2	0.1
Control (Not Sprayed)				
206	18		0.1	
141	9		0.1	
153	12		0.1	
Av. 167	13		0.1	
7 Days after Spraying with 2,4-D				
146	25		0.2	
134	10		0.1	
192	5		<0.1	
166	3		<0.1	
224	6		<0.1	
157	23		0.1	
135	5		<0.1	
196	8		<0.1	
190	8		<0.1	
157	14		0.1	
178	7		<0.1	
165	5		<0.1	
204	4		<0.1	
182	5		<0.1	
169	14		<0.1	
Av. 173	9		0.1	

[a] Apparent less control.

Recovery of 2,4-D from Fortified Lemon Pulp. Individual lemons were cut up and ground in a Waring Blendor with acetone and 2 ml. of $1 \times 10^{-3}M$ 2,4-D in the form of isopropyl ester dissolved in acetone (442-μg. acid equivalent). Recovery of the 2,4-D proceeded according to the above outline for determination of total 2,4-D.

The apparent 2,4-D found in seven lemons was 100, 97, 101, 97, 113, 106, and 89% of the amount added to the pulp. Nonfortified fruit gave an average reading of 12.4 μg. of 2,4-D per fruit. In subtracting the latter as a background reading for the fortified fruit, the recovery for the seven fruits was 96, 93, 97, 109, 102, and 85%, or an average of 96%.

Results

2,4-D Residue in Oranges. Washington Navel oranges are ordinarily sprayed with 8 p.p.m. of 2,4-D acid equivalent to reduce preharvest fruit drop. However, in this experiment, five trees were sprayed with 20 p.p.m. to provide samples for residue analysis. To serve as controls, some of the oranges were picked before the spray was applied. Immediately after the spray was applied to each tree, three dripping wet oranges were picked and rinsed with acetone to strip the 2,4-D from the surface of the fruit for analysis (Table I). Rinses of additional oranges were obtained 7 days after spraying.

The freshly sprayed oranges had an average apparent 2,4-D residue of 32 μg. per fruit. After subtraction of a reading equivalent to 13 μg. found for nonsprayed fruit, the corrected value was 19 μg. of 2,4-D per fruit, which represented 0.1 p.p.m. on the basis of fruit weight. Seven days later, the apparent 2,4-D per fruit was 9 μg., a value slightly lower than that for nonsprayed fruit the week before. From the preliminary experiment on the evaporation of the isopropyl ester of 2,4-D it would be expected that all the ester should have evaporated in less than 1 week. A 2,4-D value less than that of the control fruit indicated that the spray may have rinsed some chloride-containing compound from the fruit during application of the 2,4-D.

Other samples of sprayed oranges were picked for tissue analysis on the day after spraying (when the fruit was dry) and again after 7 days. Three 10-orange samples were obtained by picking six oranges from each of the five trees. Control fruit was picked before spraying. A longitudinal wedge of tissue equal to about $^1/_8$ of a fruit was removed from each of 10 fruits for a determination of 2,4-D. Second wedges were obtained from the same fruits for duplicates. On the seventh day, the fruits were sampled in the same manner, except that four wedges were obtained from each fruit. One set of duplicates was used for total 2,4-D and the other set for analysis involving the separation of acid and ester fractions.

The analysis of fruit tissue, the day after spraying, showed an average apparent 2,4-D acid equivalent content of 26 μg., or a corrected content of 15 μg. (Table II). The 15 μg. represented 0.1 p.p.m. of the fruit tissue, and were about two thirds as much as that recovered from the surface of freshly sprayed fruit, indicating losses by dripping of the spray from the fruit, evaporation of the ester, and probably some metabolism of the absorbed 2,4-D.

Seven days after spraying, the recovery of total 2,4-D showed that the average apparent 2,4-D acid equivalent had

Table II. Recovery of 2,4-D from Duplicates of Composite Samples of Washington Navel Oranges Sprayed with 20 P.P.M. of 2,4-D Acid Equivalent as the Isopropyl Ester

(Approximately one eighth of each of 10 fruits per determination)

Sample	Fruit Weight, Grams	2,4-D Acid Equivalent Recovered			
		μg.		P.P.M. in Fruit	
		Apparent	Corrected[a]	Apparent	Corrected[a]
1 Day after Spraying with 2,4-D					
1a	253	30	19	0.1	0.1
b	209	29	18	0.1	0.1
2a	223	28	17	0.1	0.1
b	202	20	9	0.1	<0.1
3a	216	26	15	0.1	0.1
b	192	22	11	0.1	0.1
Av.	216	26	15	0.1	0.1
Control (not sprayed)					
1a	222	7		<0.1	
b	229	9		<0.1	
2a	215	6		<0.1	
b	246	19		0.1	
3a	219	16		0.1	
b	239	8		<0.1	
Av.	228	11		<0.1	
7 Days after Spraying with 2,4-D[b]					
1a	...	..	..	...	...
b	205	17	6	0.1	<0.1
2a	227	21	10	0.1	<0.1
b	207	18	7	0.1	<0.1
3a	219	34	23	0.2	0.1
b	209	13	2	0.1	<0.1
Weighted Av.	212	20	9	0.1	<0.1
7 Days after Spraying					
Acid Fraction					
1a	205	8	6	<0.1	<0.1
b	213	2	0	<0.1	0.0
2a	210	17	15	0.1	0.1
b	214	17	15	0.1	0.1
3a	210	15	13	0.1	0.1
b	...	..	..	...	...
Weighted Av.	210	12	10	0.1	<0.1
Ester Fraction					
1a	205	11	0	0.1	0.0
b	213	11	0	0.1	0.0
2a	210	8	−3	<0.1	0.0
b	...	..	..	...	...
3a	210	27	16	0.1	0.1
b	...	..	..	...	...
Weighted Av.	210	15	4	0.1	<0.1
Control (not sprayed)					
Acid Fraction					
1	214	1		Trace	
2	...	..		...	
3	214	2		Trace	
Av.	214	2		Trace	
Ester Fraction					
1	214	15		0.1	
2	221	6		<0.1	
3	214	11		0.1	
Av.	216	11		0.1	

[a] Apparent less control.
[b] Same control as above.

Table III. Recovery of 2,4-D from Individual Lemons Dipped in Water–Wax Emulsions with or without 500 P.P.M. of 2,4-D Acid Equivalent as the Isopropyl Ester

Fruit Weight, Grams	2,4-D Acid Equivalent Recovered			
	μg.		P.P.M. in Fruit	
	Apparent	Corrected[a]	Apparent	Corrected[a]
	On Day of Treatment[b]			
154	164	148	1.1	1.0
125	142	126	1.1	1.0
128	91	75	0.7	0.6
108	97	81	0.9	0.8
110	121	105	1.1	0.9
Av. 125	123	107	1.0	0.9
	Control Fruit[c]			
140	19		0.1	
132	13		0.1	
102	14		0.1	
125	18		0.1	
113	18		0.1	
Av. 122	16		0.1	
	2 Days after Treatment[b]			
Acid Fraction				
106	73	50	0.7	0.5
105	233	210	2.2	2.0
102	236	213	2.3	2.1
112	131	108	1.2	0.9
132	85	62	0.6	0.5
110	71	48	0.6	0.4
Av. 111	138	115	1.2	1.0
Ester Fraction				
106	25	19	0.2	0.2
105	27	21	0.3	0.2
102	22	16	0.2	0.2
112	19	13	0.2	0.1
132	14	8	0.1	0.1
110	13	7	0.1	0.1
Av. 111	20	14	0.2	0.1
	Control Fruit[c]			
Acid Fraction				
127	10		0.1	
125	8		0.1	
107	28		0.3	
109	45		0.4	
Av. 117	23		0.2	
Ester Fraction				
127	8		0.1	
125	4		<0.1	
107	4		<0.1	
109	6		0.1	
Av. 117	6		0.1	

[a] Apparent less control.
[b] Treated fruit with 500 p.p.m. of 2,4-D in wax emulsion.
[c] No 2,4-D in wax emulsion.

dropped from 26 to 20 μg. for essentially equal weights of fruit tissue. In applying the same correction of 11 μg. for nonsprayed fruit, a value of 9 μg. or <0.1 p.p.m. was obtained.

The other samples of fruit analyzed 7 days after spraying were separated into acid and ester fractions. The acid fraction from sprayed fruit showed a corrected average of 10 μg. of 2,4-D per fruit as compared with 4 μg. for the ester. The high and variable background in the ester fraction precluded any reliable estimate of the degree of hydrolysis of the ester in these samples.

2,4-D Residues in Lemons. Freshly picked lemons were washed in water and detergent, rinsed in clear water, and dipped in 1% of wax emulsion plus 1% of soda ash either with or without 500 p.p.m. of 2,4-D acid equivalent in the form of a commercial isopropyl ester formulation. The lemons were washed and dipped and allowed to surface-dry before they were ground up and extracted with acetone.

Total apparent 2,4-D acid equivalent in the 2,4-D-treated lemons averaged 123 μg. as compared with 16 μg. in the control fruit (Table III). Based on fruit weight, the apparent concentration of 2,4-D averaged 1.0 p.p.m., or after allowing for background reading, a concentration of 0.9 p.p.m.

In another experiment with lemons, the fruit was dipped in wax emulsion with or without 2,4-D as before, but placed in storage at 14° to 15° C. until analyzed 2 days after treatment. The 2,4-D was separated into acid and ester fractions.

The results (Table III) showed that the treated fruit had an average, apparent 2,4-D acid equivalent content of 138 μg. per fruit in the acid fraction or a corrected value of 115 μg. which equaled 1.0 p.p.m. of the fruit weight. In the ester fraction, the average, apparent 2,4-D content was 20 μg. or 14 μg. corrected, which was equivalent to 0.1 p.p.m. of the fruit weight. These data indicated that 90% of the 2,4-D was hydrolyzed 2 days after application.

Three lemons, treated with 500 p.p.m. of 2,4-D 90 days before analysis, showed a total apparent 2,4-D content of 14, 7, and 6 μg. These values were equivalent to about 0.1 p.p.m. of 2,4-D in the fruit and were no higher than values found in nontreated fruit.

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