orange and turned a bright pink color. Silica from rice sheath adsorbed only enough to show a slight trace of pink color.

The adsorptive capacities of the two forms of silica were quite different and indicated that the rice silica was not porous like the silica gel.

The specific gravity of the rice sheath silica at 20° C. was 2.257. This is at the upper end of the opal range. The corresponding value for the silica gel was 2.102. This was also good evidence that the rice silica was the least porous.

These studies indicate that rice silica is not exactly the same as ordinary silica gel and that it would be better to classify it as biogenetic opal. This is in line with the common practice of classifying amorphous plant silica as opal.


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### Literature Cited


(1) Beavers, A. H., Stephens, I., *Soil Sci.* **86,** 1 (1958).
(2) Bonart, R., Hoseman, R., Motzkus, F., Ruch, H., *Norelco Reptr.* **7,** No. 3, 81 (1960).
(3) Engel, W., *Planta* **41,** 358 (1953).
(4) Franks, P. C., Swineford, A., *J. Sediment. Petrol.* **29,** No. 2, 189–93 (1959).
(5) Ishibashi, H., *J. Sci. Soil Manure, Japan* **10,** 244 (1936).
(6) Ishibashi, H., Kawano, M., *Bull. Fac. Agr. Yamaguti Univ.* **9,** 917–22 (1953).
(7) Lanning, F. C., *Proc. Am. Soc. Hort. Sci.* **76,** 349–58 (1960).
(8) *Ibid.*, **77,** 367–71 (1961).
(9) Lanning, F. C., Ponnaiya, B. W. X., Crumpton, C. F., *Plant Physiol.* **33,** 339–43 (1958).
(10) Liu, S-L., Ho, C-H., *J. Chinese Chem. Soc. Taiwan* **6,** 141–53 (1960).
(11) Oota, M., *Bull. Fac. Lib. Arts Educ. Yamanashi Univ.* **5,** 183 (1954).
(12) Ponnaiya, B. W. X., *Madras Univ. J.* **XXI,** Sect. B, No. 2 (1951).
(13) Smithson, F., *Nature* **178,** 107 (1956).
(14) Swineford, A., Franks, P. C., *Soc. Econ. Paleontologists Mineralogists Spec. Publ.* **7,** 111–20 (1959).
(15) Walton, H. F., "Inorganic Preparations," p. 114, Prentice-Hall, New York, 1948.
(16) Weiss, A., *Beitr. Silikose-Forsch. Sonderband* **2,** 303–16 (1956).
(17) Yoshida, S., Ohnishi, Y., Kitagishi, K., *Soil Plant Food Tokyo* **5,** 23–7 (1959).



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**2,4-D RESIDUES**

# Residues in Stored Lemons Treated with Various Formulations of 2,4-D


**LOUIS C. ERICKSON, B. L. BRANNAMAN, and CHARLES W. COGGINS, Jr.**

**Department of Biochemistry and Department of Horticultural Science, University of California, Riverside, Calif.**



**Analysis of lemons for 2,4-D residues showed a small amount of ester-like residue whether the fruit was treated with isopropyl ester or various salt formulations. The use of carboxyl-$C^{14}$-labeled 2,4-D acid indicated that part of the 2,4-D reacted with some plant constituent to produce an ester-like complex. A $C^{14}$-label in the isopropyl group provided evidence that all of the isopropyl ester in the cells was hydrolyzed and that any ester-like residue was synthesized in vivo.**


It was shown previously (*3*) that 2,4-D (2,4-dichlorophenoxyacetic acid) applied to citrus in the form of isopropyl ester was recovered in what appeared to be both acid and ester fractions. The acid fraction partitioned to organic solvents at a low pH and to water in a neutral solution. The fraction considered to be the ester comprised only a small part of the residue but was found to be water-insoluble in a neutral solution. Alkaline hydrolysis yielded the acid.

Although Crafts (*2*) found that hydrolysis of isopropyl 2,4-D occurred in barley leaves, the evidence did not indicate the extent of hydrolysis. Previous data from the study of citrus indicated that some of the residue might remain as the isopropyl ester.

Further investigation was undertaken to determine the amount and nature of residues left by various salt forms of 2,4-D. $C^{14}$-labeled 2,4-D was also used to determine the degree of hydrolysis of the isopropyl ester. Results of these investigations are presented here.

### Procedure

Lemons were washed in a commercial-type washer. The wash water contained 0.5% soap and 2% soda ash; it was kept at 110° F., and the fruit remained submerged for 2 minutes. The final rinse in the washing process consisted of a wax emulsion containing 0.5% of Sunkist water-wax. The 2,4-D was added to the wax emulsion in forms described in the separate experiments. Each treatment contained more than 400 fruit known commercially as light green.

Analyses for 2,4-D residues of individual fruits taken at random were made initially and after various periods of storage by using a Dohrmann microcoulometric gas chromatograph and a method previously described (*3*). The fruit remaining at the end of the experiments was inspected for soundness and general appearance.

Additional experiments were made with $C^{14}$-labeled 2,4-D. The $C^{14}$-label was present in the carboxyl position of both isopropyl ester and acid forms of the growth regulator and also in the isopropyl group (1,3 position) of another preparation of the isopropyl ester. Fruit in these experiments was hand-washed and treated with 2,4-D over a small marked area of the fruit surface by application with a micropipet. The 2,4-D compounds were dissolved in acetone to facilitate application. The treated fruit was kept in large closed jars and aerated with humidified air. After storage, the treated portion of the peel was grated, frozen, and extracted with acetone for about 4 hours in a Bailey-Walker extractor. The acetone was evaporated and the residue dissolved in ether, filtered, and finally concentrated to about 0.5 ml. before

## Table I. Analysis of 2,4-D Residues on Lemons

(Washed September 20, 1961)

| Fruit Weight, Grams | 2,4-D Acid Fraction μg. | 2,4-D Acid Fraction P.P.M. | 2,4-D Ester Fraction μg. | 2,4-D Ester Fraction P.P.M. | Total 2,4-D, P.P.M. |
|---|---|---|---|---|---|
| *1 Day after Washing* | | | | | |
| Control—no 2,4-D applied in wax | | | | | |
| 114 | 0 | 0.00 | 0.6 | 0.01 | 0.01 |
| 119 | 0 | 0.00 | 0.9 | 0.01 | 0.01 |
| 115 | 0.5 | 0.00 | 7.1 | 0.06 | 0.06 |
| 110 | 0.9 | 0.01 | 5.1 | 0.05 | 0.06 |
| Av. | 0.4 | 0.00 | 3.4 | 0.03 | 0.03 |
| 500 P.P.M. 2,4-D as Weedone 638, emulsifiable acid | | | | | |
| 92 | 36.3 | 0.39 | 12.8 | 0.14 | 0.53 |
| 115 | 23.5 | 0.20 | 6.2 | 0.05 | 0.25 |
| 101 | 42.1 | 0.42 | 6.7 | 0.07 | 0.49 |
| 105 | 32.2 | 0.31 | 8.2 | 0.08 | 0.39 |
| Av. | 33.5 | 0.33 | 8.5 | 0.09 | 0.42 |
| 500 P.P.M. 2,4-D as Esteron 44, isopropyl ester | | | | | |
| 95 | 90.3 | 0.95 | 10.7 | 0.11 | 1.06 |
| 119 | 18.8 | 0.16 | 5.5 | 0.05 | 0.21 |
| 113 | 60.0 | 0.53 | 11.2 | 0.10 | 0.63 |
| 114 | 29.9 | 0.26 | 8.3 | 0.07 | 0.33 |
| Av. | 49.8 | 0.48 | 8.9 | 0.08 | 0.56 |
| 500 P.P.M. 2,4-D as sodium salt | | | | | |
| 135 | 35.3 | 0.26 | 2.1 | 0.02 | 0.28 |
| 123 | 47.3 | 0.38 | 1.0 | 0.01 | 0.39 |
| 119 | 32.2 | 0.27 | 0.6 | 0.01 | 0.28 |
| 117 | 29.2 | 0.25 | 1.3 | 0.01 | 0.26 |
| Av. | 36.0 | 0.29 | 1.3 | 0.01 | 0.30 |
| *7 Days after Washing* | | | | | |
| Control—no 2,4-D applied in wax | | | | | |
| 113 | 0.6 | 0.01 | 1.0 | 0.01 | 0.02 |
| 124 | 0.3 | 0.00 | 0.5 | 0.00 | 0.00 |
| 113 | 1.2 | 0.01 | 1.1 | 0.01 | 0.02 |
| 124 | 1.6 | 0.01 | 0.8 | 0.01 | 0.02 |
| Av. | 0.9 | 0.01 | 0.9 | 0.01 | 0.02 |
| 500 P.P.M. 2,4-D as Weedone 638, emulsifiable acid | | | | | |
| 123 | 23.5 | 0.19 | 7.1 | 0.06 | 0.25 |
| 98 | 29.8 | 0.30 | 6.6 | 0.07 | 0.37 |
| 122 | 26.5 | 0.22 | ... | ... | ... |
| 105 | 35.0 | 0.33 | 3.4 | 0.03 | 0.36 |
| Av. | 28.7 | 0.26 | 5.7 | 0.05 | 0.33 |
| 500 P.P.M. 2,4-D as Esteron 44, isopropyl ester | | | | | |
| 126 | 33.5 | 0.27 | 5.2 | 0.04 | 0.31 |
| 113 | 22.5 | 0.20 | 3.3 | 0.03 | 0.23 |
| 99 | 19.8 | 0.20 | 5.2 | 0.05 | 0.25 |
| 132 | 29.3 | 0.22 | 8.0 | 0.06 | 0.28 |
| Av. | 26.3 | 0.22 | 5.4 | 0.05 | 0.27 |
| 500 P.P.M. 2,4-D as sodium salt | | | | | |
| 134 | 52.5 | 0.39 | 2.3 | 0.02 | 0.41 |
| 103 | 30.5 | 0.30 | 1.9 | 0.02 | 0.32 |
| 122 | 59.2 | 0.49 | 4.6 | 0.04 | 0.53 |
| 101 | 26.6 | 0.26 | 3.8 | 0.04 | 0.30 |
| Av. | 42.2 | 0.36 | 3.2 | 0.03 | 0.39 |
| *14 Days after Washing* | | | | | |
| 500 P.P.M. 2,4-D as Weedone 638, emulsifiable acid | | | | | |
| 119 | 44.3 | 0.37 | 8.4 | 0.07 | 0.44 |
| 104 | 20.0 | 0.19 | 7.8 | 0.08 | 0.27 |
| 97 | 27.6 | 0.28 | 4.9 | 0.05 | 0.33 |
| 118 | 32.0 | 0.27 | 9.2 | 0.08 | 0.35 |
| Av. | 31.0 | 0.28 | 7.6 | 0.07 | 0.35 |
| 500 P.P.M. 2,4-D as Esteron 44, isopropyl ester | | | | | |
| 101 | 33.2 | 0.33 | 7.9 | 0.08 | 0.41 |
| 112 | 28.0 | 0.25 | 8.0 | 0.07 | 0.32 |
| 96 | 21.6 | 0.23 | 8.6 | 0.09 | 0.32 |
| 105 | 9.0 | 0.09 | 10.4 | 0.10 | 0.19 |
| Av. | 23.0 | 0.23 | 8.7 | 0.09 | 0.31 |
| 500 P.P.M. 2,4-D as sodium salt | | | | | |
| 114 | 52.5 | 0.46 | 4.7 | 0.04 | 0.50 |
| 120 | 51.0 | 0.43 | 9.3 | 0.08 | 0.51 |
| 125 | 52.0 | 0.42 | 7.0 | 0.06 | 0.48 |
| 110 | 47.5 | 0.43 | 9.7 | 0.09 | 0.52 |
| Av. | 50.8 | 0.44 | 7.7 | 0.07 | 0.50 |
| *42 Days after Washing* | | | | | |
| Control—no 2,4-D applied in wax | | | | | |
| 113 | 1.6 | 0.01 | 1.2 | 0.01 | 0.02 |
| 123 | 0.5 | 0.00 | 1.1 | 0.01 | 0.01 |
| 101 | 1.1 | 0.01 | 1.0 | 0.01 | 0.02 |
| 102 | 0 | 0 | 0.6 | 0.01 | 0.01 |
| Av. | 0.8 | 0.01 | 1.0 | 0.01 | 0.02 |
| 500 P.P.M. 2,4-D as Weedone 638, emulsifiable acid | | | | | |
| 116 | 23.5 | 0.20 | 7.5 | 0.06 | 0.26 |
| 118 | 30.7 | 0.26 | 8.0 | 0.07 | 0.33 |
| 95 | 8.4 | 0.09 | 3.3 | 0.03 | 0.12 |
| 117 | 11.7 | 0.12 | 3.7 | 0.03 | 0.15 |
| Av. | 18.6 | 0.17 | 5.6 | 0.05 | 0.22 |
| 500 P.P.M. 2,4-D as Esteron 44, isopropyl ester | | | | | |
| 138 | 32.5 | 0.24 | 8.5 | 0.06 | 0.30 |
| 107 | 18.7 | 0.17 | 5.1 | 0.05 | 0.22 |
| 124 | 18.9 | 0.15 | 7.6 | 0.06 | 0.21 |
| 128 | 12.0 | 0.09 | 7.6 | 0.06 | 0.15 |
| Av. | 20.5 | 0.16 | 7.2 | 0.06 | 0.22 |
| 500 P.P.M. 2,4-D as sodium salt | | | | | |
| 109 | 16.2 | 0.15 | 2.5 | 0.02 | 0.17 |
| 129 | 42.6 | 0.33 | 2.0 | 0.02 | 0.35 |
| 101 | 35.4 | 0.35 | 0.3 | 0.00 | 0.35 |
| 95 | 38.8 | 0.41 | 1.8 | 0.02 | 0.43 |
| Av. | 33.3 | 0.31 | 1.7 | 0.02 | 0.33 |

spotting on filter paper for chromatography. The chromatograms were developed for 8 hours with isopropanol, ammonium hydroxide, and water (10:1:1). Radioautographs were made by contact exposure of x-ray film for 3 weeks.

### Results

**Isopropyl Ester, Emulsifiable Acid, and Sodium Salt of 2,4-D.** Isopropyl ester (Esteron 44), emulsifiable acid (Weedone 638), and sodium salt of 2,4-D were applied in the wax emulsion at 500 p.p.m. acid equivalent. Control fruits were treated with wax emulsion only. Initial samples of four individual fruits from the controls and from each of the treatments were analyzed for 2,4-D 1 day after treatment. Additional analyses were made after 7, 14, and 42 days of storage.

Although the average amount of the ester fraction found did not exceed 0.09 p.p.m. in any treatment on any of the dates of sampling (Table I) and thus may not have been the maximum concentration of complex which formed, it is significant that ester-like residues were found when the 2,4-D was applied as sodium salt or as an emulsifiable acid preparation, as well as when it was applied as the isopropyl ester. The amount of ester-like fraction recovered from fruit treated with the sodium salt of 2,4-D was 0.07 p.p.m. after 14 days of storage.

## Table II. Condition of Lemons after 3 Months of Storage

(Washed September 20, inspected December 21, 1961)

| 2,4-D Treatment | Total Fruit | Black Buttons, % | Alternaria, % | Other Decay, % |
|---|---|---|---|---|
| None | 109 | 4.6 | 0.0 | 0.0 |
| | 113 | 8.0 | 0.0 | 0.9 |
| | 106 | 3.8 | 0.0 | 2.8 |
| | 117 | 5.1 | 0.9 | 0.9 |
| Av. | | 5.4 | 0.2 | 1.2 |
| Emulsifiable acid (Weedone 638) | | | | |
| 500 P.P.M. | 114 | 0.0 | 0.0 | 0.0 |
| | 105 | 1.0 | 1.0 | 0.0 |
| | 111 | 0.0 | 0.0 | 0.9 |
| | 112 | 1.8 | 0.9 | 0.0 |
| Av. | | 0.7 | 0.5 | 0.2 |
| Isopropyl ester (Esteron 44) | | | | |
| 500 P.P.M. | 118 | 0.8 | 0.8 | 1.7 |
| | 113 | 0.9 | 0.0 | 0.0 |
| | 109 | 0.0 | 0.0 | 0.0 |
| | 106 | 0.9 | 0.9 | 1.9 |
| Av. | | 0.7 | 0.4 | 0.9 |
| Sodium salt | | | | |
| 500 P.P.M. | 113 | 0.0 | 0.0 | 0.0 |
| | 115 | 0.9 | 0.0 | 0.0 |
| | 107 | 0.0 | 0.0 | 0.9 |
| | 111 | 0.0 | 0.0 | 0.0 |
| Av. | | 0.2 | 0.0 | 0.2 |

## Table III. Analysis of 2,4-D Residues on Lemons

(Washed November 6, 1961)

| Fruit Weight, Grams | 2,4-D Acid Fraction µg. | 2,4-D Acid Fraction P.P.M. | 2,4-D Ester Fraction µg. | 2,4-D Ester Fraction P.P.M. | Total 2,4-D, P.P.M. |
|---|---|---|---|---|---|
| *1 Day after Washing* | | | | | |
| Control—no 2,4-D in wax emulsion | | | | | |
| 109 | 0.7 | 0.01 | 3.4 | 0.03 | 0.04 |
| 116 | 0.8 | 0.01 | 0.8 | 0.01 | 0.02 |
| 112 | 0.7 | 0.01 | 0.0 | 0.00 | 0.01 |
| 110 | 0.8 | 0.01 | 2.0 | 0.02 | 0.03 |
| Av. | 0.8 | 0.01 | 1.6 | 0.02 | 0.03 |
| 500 P.P.M. 2,4-D as diethanolamine salt | | | | | |
| 105 | 2.8 | 0.03 | 0.8 | 0.01 | 0.04 |
| 120 | 34.2 | 0.29 | 0.8 | 0.01 | 0.30 |
| 135 | 57.7 | 0.43 | 0.6 | 0.00 | 0.43 |
| 109 | 43.3 | 0.40 | 0.5 | 0.00 | 0.40 |
| Av. | 34.5 | 0.29 | 0.7 | 0.01 | 0.29 |
| 500 P.P.M. 2,4-D as triethanolamine salt | | | | | |
| 104 | 11.6 | 0.11 | 0.8 | 0.01 | 0.12 |
| 130 | 33.1 | 0.25 | 0.4 | 0.00 | 0.25 |
| 98 | 32.4 | 0.33 | 0.0 | 0.00 | 0.33 |
| 115 | 34.6 | 0.30 | 1.5 | 0.01 | 0.31 |
| Av. | 27.9 | 0.25 | 0.7 | 0.01 | 0.25 |
| *14 Days after Washing* | | | | | |
| Control—no 2,4-D in wax emulsion | | | | | |
| 120 | 1.2 | 0.01 | 0.5 | 0.00 | 0.01 |
| 127 | 0.2 | 0.00 | 0.5 | 0.00 | 0.00 |
| 105 | 0.2 | 0.00 | 0.5 | 0.00 | 0.00 |
| 106 | 1.0 | 0.01 | 0.5 | 0.00 | 0.01 |
| Av. | 0.7 | 0.01 | 0.5 | 0.00 | 0.01 |
| 500 P.P.M. 2,4-D as diethanolamine salt | | | | | |
| 115 | 33.2 | 0.29 | 2.1 | 0.02 | 0.31 |
| 121 | 33.4 | 0.28 | 2.8 | 0.02 | 0.30 |
| 113 | 18.4 | 0.16 | 2.7 | 0.02 | 0.18 |
| 111 | 37.4 | 0.34 | 1.5 | 0.01 | 0.35 |
| Av. | 30.6 | 0.27 | 2.3 | 0.02 | 0.29 |
| 500 P.P.M. 2,4-D as triethanolamine salt | | | | | |
| 116 | 51.4 | 0.44 | 3.6 | 0.03 | 0.47 |
| 114 | 32.6 | 0.29 | 3.1 | 0.03 | 0.32 |
| 116 | 29.3 | 0.25 | 3.4 | 0.03 | 0.28 |
| 113 | 49.4 | 0.44 | 3.1 | 0.03 | 0.47 |
| Av. | 40.7 | 0.36 | 3.3 | 0.03 | 0.39 |

## Table IV. Condition of Lemons after 4 Months of Storage

(Washed November 6, inspected March 6, 1962)

| 2,4-D Treatment | Total Fruit | Black Buttons, % | Alternaria, % | Other Decay, % |
|---|---|---|---|---|
| None | 101 | 20.8 | 5.0 | 1.0 |
| | 110 | 19.1 | 2.7 | 1.8 |
| | 105 | 28.6 | 3.8 | 0.0 |
| | 112 | 29.5 | 6.3 | 0.0 |
| Av. | | 24.5 | 4.5 | 0.7 |
| Diethanolamine salt | | | | |
| 500 P.P.M. | 114 | 0.9 | 0.9 | 0.0 |
| | 109 | 0.9 | 0.9 | 0.0 |
| | 101 | 0.0 | 1.0 | 0.0 |
| | 108 | 0.0 | 0.0 | 1.9 |
| Av. | | 0.5 | 0.7 | 0.5 |
| Triethanolamine salt | | | | |
| 500 P.P.M. | 108 | 0.9 | 0.9 | 0.9 |
| | 110 | 0.0 | 0.0 | 0.0 |
| | 106 | 0.0 | 0.9 | 1.9 |
| | 104 | 0.0 | 0.0 | 0.0 |
| Av. | | 0.2 | 0.5 | 0.7 |

An inspection of the lemons was made after 3 months of storage (Table II). Counts were made separately in each half of the partitioned duplicate boxes, thus providing four subsamples per treatment. In this lot of fruit, the natural storage life was fairly long and so the differences between treatments were not great. However, the appearance of black buttons was becoming noticeable in the controls at the end of 3 months.

**Diethanolamine and Triethanolamine Salts of 2,4-D.** The diethanolamine and triethanolamine salts of 2,4-D were applied to lemons in wax emulsion at a concentration of 500 p.p.m. acid equivalent. Analyses for 2,4-D residues were made after 1 and 14 days. Again, as when treated with the sodium salt, there was a tendency for more ester fraction to be present after 14 days of storage than initially (Table III).

In the inspection of fruit made after 4 months of storage (Table IV), the controls averaged 24.5% black buttons


Figure 1. Chromatograms of extracts of lemon peel after applying isopropyl ester of 2,4-D labeled with $C^{14}$ in the carboxyl position

(a) isopropyl 2,4-D diluted with acetone; (b) 2,4-D acid dissolved in acetone; (c) isopropyl 2,4-D added to control peel extract before spotting; (d) 2,4-D acid added to control peel extract before spotting; (e–h) lemon peel extracts 14 days after applying isopropyl 2,4-D

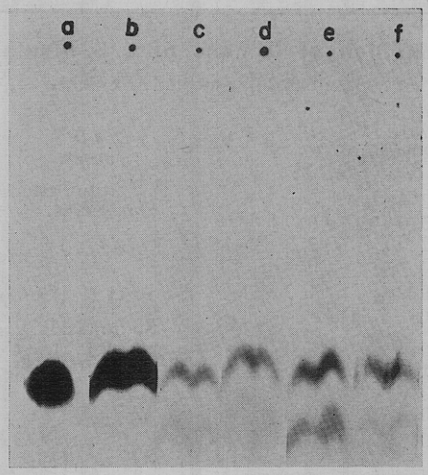


Figure 2. Chromatograms of extracts of lemon peel after applying 2,4-D acid labeled with $C^{14}$ in the carboxyl position

(a) 2,4-D acid dissolved in acetone; (b) 2,4-D acid dissolved in acetone and added to peel extract of control fruit before spotting; (c–f) lemon peel extracts 14 days after applying 2,4-D acid

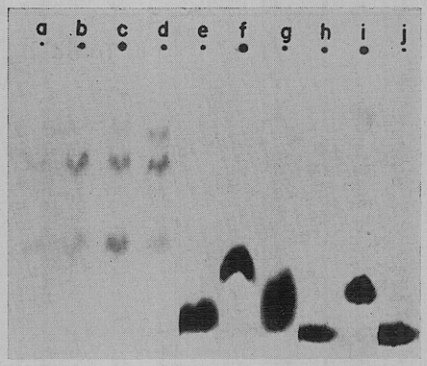


Figure 3. Chromatograms of extracts of lemon peel after applying isopropyl ester of 2,4-D labeled with $C^{14}$ in the isopropyl group (1:3)

(a–d) lemon peel extracts 7 days after treatment with 2,4-D ester labeled in the isopropyl group; (e) isopropyl ester (isopropyl 1:3 label) added to control peel extract before spotting; (f) 2,4-D acid (carboxyl label) added to control peel extract before spotting; (g) isopropyl ester (carboxyl label) added to control peel extract before spotting; (h) isopropyl ester (isopropyl 1:3 label) dissolved in acetone; (i) 2,4-D acid (carboxyl label) dissolved in acetone; (j) isopropy ester (carboxyl label) dissolved in acetone

and 4.5% *Alternaria* rot, while the diethanolamine and triethanolamine salts of 2,4-D resulted in less than 1% black buttons and *Alternaria*.

**Isopropyl Ester of 2,4-D Labeled with $C^{14}$ in the Carboxyl Position.** The results of extracting and chromatographing carboxyl-$C^{14}$–labeled isopropyl 2,4-D 14 days after application to lemons are shown in Figure 1. Eight separate strips of paper were developed simultaneously by descending chromatography. $C^{14}$-carbonate marks were then applied 1 inch above the sites of spotting. All of the strips were exposed to a single sheet of x-ray film for 3 weeks before development.

The lemon peel extracts exhibited two prominent radioactive spots in the chromatograms—the more prominent corresponded with the location of 2,4-D acid, while the less prominent was intermediate between the location of the acid and the isopropyl ester, but could not definitely be ruled out as being the latter on this criterion alone.

**2,4-D Acid Labeled with $C^{14}$ in the Carboxyl Position.** Lemons were treated with 2,4-D acid labeled with $C^{14}$ in the carboxyl position and the peels extracted after 14 days. The results of this experiment showed (Figure 2) that some of the 2,4-D acid applied to the fruit was altered in a manner to give an $R_f$ different from that of the 2,4-D acid but similar to that of the secondary spots when isopropyl ester was applied. This indicated the probability that whatever ester-like material was found in previous residue analyses was fruit-synthesized after the original isopropyl ester had been hydrolyzed.

The supposed ester spots were cut from the filter paper, eluted with acetone, and rechromatographed after alkaline hydrolysis of the eluate. A radioactive spot then appeared at the $R_f$ of 2,4-D acid, indicating that the material was readily converted to 2,4-D by hydrolysis, in the manner of an ester.

**Isopropyl Ester of 2,4-D Labeled with $C^{14}$ in the Isopropyl Group (1:3).** Application of isopropyl-labeled 2,4-D ester was made as in the previous experiments where carboxy-labeled material was used. The peel was extracted 7 days after treatment. The four chromatograms on the left in Figure 3 (*a* to *d*), which represent the extracts from fruit peel, show that the isopropyl label did not chromatograph in the location of the isopropyl ester and therefore indicate that the 2,4-D ester was completely hydrolyzed in the fruit.

## Discussion

In the present investigation, no attempt was made to study the metabolism of 2,4-D in lemon fruits. The primary objective was to determine whether any isopropyl ester of 2,4-D remained as a residue in the fruit after a period of storage. The evidence indicated that the isopropyl ester did indeed hydrolyze in the tissues as was shown earlier by Crafts (*2*). Moreover, the hydrolysis was found to be complete.

An interesting aspect of the determination of 2,4-D residues by a method previously published (*3*) is that the separation of acid and ester fractions, while effective, does not determine the nature of the ester-like fraction. Direct chromatography of the extracted residue without the hydrolysis and synthesis of the methyl ester would probably give some indication of the volatility of the residue. Yip (*4*), in a study of the residence time of various esters of 2,4-D and other chlorinated phenoxy alkyl acids in the Dohrmann microcoulometric gas chromatograph, found that separation of some of the esters was possible. His study was not carried to the stage of recovering residues from sprayed plants, however, and so it is not possible to evaluate this procedure for the identification of residue fractions.

It is known that 2,4-D will form complexes in plants (*1*), but beyond the recovery and separation of numerous fractions, the metabolic steps taken by 2,4-D have not been completely explored. In addition to the obvious value that a thorough understanding of the metabolism of 2,4-D will have in the area of residue problems, it will undoubtedly contribute much to an understanding of the mode of action of this and other plant growth regulators.

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## Literature Cited

(1) Bach, M. K., *Plant Physiol.* **36,** 558 (1961).
(2) Crafts, A. S., *Weeds* **8,** 19 (1960).
(3) Erickson, L. C., Hield, H. Z., J. Agr. Food Chem. **10,** 204 (1962).
(4) Yip, G., *J. Assoc. Offic. Agr. Chemists* **45,** 367 (1962).

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