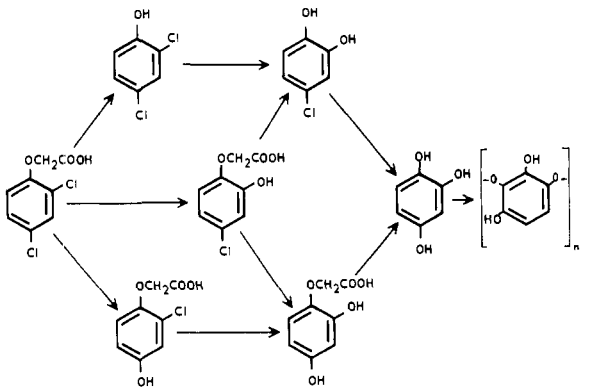

Figure 3. Proposed mechanism of 2,4-D photodecomposition

The reported decrease in pH of the 2,4-D solution upon irradiation (*2*) may be explained by the formation of 2 moles of hydrochloric acid for each mole of 2,4-D decomposed. Although one of these is neutralized by reaction with the sodium salt of the organic acid, the carboxyl group thus liberated also contributes to the total acidity. Furthermore, the apparent oxidation of the glycolic acid resulting from cleavage of the ether bond would provide further basis for the low observed pH if oxalic acid were assumed to be a final product.

Sunlight appears to produce many of the same qualitative effects as does ultraviolet light in the laboratory, although the number of detectable products is greater under the artificial illumination. The failure to detect 2,4-dichlorophenol may be due to rapid volatilization under outdoor conditions, or it may indicate that less energy is required for replacement of halogen by hydroxyl than for rupture of the aralkyl ether linkage. However, the fact that humic acid also is observed in the sunlight-irradiated mixture suggests that the ether bond, too, eventually is broken. Based on the amount of recovered 2,-4-D and 2,4-dichlorophenol, at least a significant part of the herbicide degraded by sunlight is converted to the brown polymer.

At very low initial concentrations of 2,4-D, such as those to be expected in polluted lakes and streams, the polymerization of 2-hydroxybenzoquinone probably would become much less important than combination with other substances dissolved in or contacting the solution. In its tautomeric form, 4-hydroxy-1,2-benzoquinone, the compound, also would be susceptible to a competing, ring-opening oxidation. However, the present experiments indicate the general course of the photodecomposition of this widely used herbicide and demonstrate the formation of one type of insoluble end product, the polyquinoid humic acids.

Acknowledgment

Herman F. Beckman and Paul Allen provided invaluable assistance and suggestions in the instrumental aspects of the research.

Literature Cited

(1) Allan, A. O., "The Radiation Chemistry of Water and Aqueous Solutions," p. 117 ff., Van Nostrand, Princeton, 1961.
(2) Aly, O. M., Faust, S. D., J. AGR. FOOD CHEM. **12,** 541 (1964).
(3) Bell, G. R., *Botan. Gaz.* **118,** 133 (1965).
(4) Brown, G. P., McCall, E. B., *J. Chem. Soc.* **1955,** p. 3681.
(5) Evans, W. C., Smith, B. S. W., *Biochem. J.* **57,** xx (1954).
(6) Hansen, J. R., Buchholtz, K. P., *Weeds* **1,** 237 (1952).
(7) Mitchell, L. C., *J. Assoc. Offic. Agr. Chemists* **44,** 643 (1961).
(8) Payne, M. G., Fults, J. L., *Science* **106,** 37 (1947).
(9) Penfound, W. T., Minyard, V., *Botan. Gaz.* **109,** 231 (1947).
(10) Ziechmann, W., Scholz, H., *Naturwissenschaften* **47,** 193 (1960).

Received for review May 5, 1966. Accepted July 21, 1966. Division of Agricultural and Food Chemistry, 149th Meeting, ACS, Detroit, Mich., April 1965. Investigation was supported in part by the U. S. Public Health Service (Grant No. EF-00306) and the U. S. Department of Agriculture Regional Project W-45.

HERBICIDES IN PEEL

A Heat-Labile Insoluble Conjugated Form of 2,4-Dichlorophenoxyacetic Acid and 2-(2,4,5-Trichlorophenoxy)propionic Acid in Citrus Peel

WILLIAM R. MEAGHER

Institute of Food and Agricultural Sciences, University of Florida Citrus Experiment Station, Lake Alfred, Fla.

IN FLORIDA, dilute sprays of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-(2,4,5-trichlorophenoxy)propionic acid (2,4,5-TP) have been found to control preharvest fruit drop of midseason oranges. In an investigation of residues of these growth regulators in oranges, a portion of the peel was converted to citrus feed. Total growth regulator residues in this product were approximately three times higher than could be accounted for by the apparent residues in the fresh fruit.

In the commercial manufacture of citrus feed, following extraction of juice, the peel is mixed with a small amount of lime, comminuted, and fed into a gas-fired dryer blast at approximately 600° F. The primary purpose of adding lime is to release water from pectin on the surface of the peel particles. The over-all pH of the peel is not significantly affected.

Nearly all of the residue occurs in the peel and since heating was the only major additional treatment the peel received, it was suspected that a form of the growth regulators normally not extractable was being liberated. A series of experiments was performed to investigate this apparent release of the growth regulator fractions under laboratory conditions.

Experimental

Methods and Materials. Samples of fresh citrus peel were prepared by compositing peel samples obtained from oranges from trees sprayed with either 20 p.p.m. (free acid basis) of 2,4-D isopropyl ester or the propylene glycol butyl ether ester of 2,4,5-TP 1 to 2 months prior to harvesting. The mixtures used varied in composition, so that actual residues reported in separate investigations are not directly comparable.

Residues of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-(2,4,5-trichlorophenoxy)propionic acid (2,4,5-TP) in orange peel, not normally extracted with acetone, are described. These conjugated fractions become available for extraction only after heating, as conjugated forms that can be hydrolyzed to the free acid form by heating in 0.5N KOH. Water is not essential for heat release of these conjugated forms from peel. Preliminary investigation points to conjugation of these growth regulators with pectin. The solubilized forms have neither acidic nor basic groups and could be sugar esters. These heat-labile fractions constitute an important part of these growth regulator residues in oranges.

All values are based on fresh weight of the peel.

2,4-D and 2,4,5-TP were determined by the method of Meagher (*5*) by electron-capture gas chromatography as butoxyethyl esters. In this procedure, peel is extracted by blending with acetone. Following removal of acetone from the filtrate by flash evaporation, fractions of the growth regulators are separated by appropriate partitioning between immiscible solvents. Free acid, ester acid (hexane-soluble), and conjugated acid (water-soluble, hexane-insoluble conjugated acid) are thus separated, and after esterification can be determined individually. Residue also may be determined directly by hydrolysis of the acetone-free extract, followed by the determination of the total soluble acid residue as one operation. Dried peel or feed and the acetone powder remaining after acetone extraction were analyzed by first hydrating with 600 ml. of water prior to extraction with acetone. Acetone powders remaining after extraction also were rehydrated prior to heating to reduce bulk and tendency to char.

Quantitative gas chromatography was conducted with a Wilken's A600C gas chromatograph equipped with an 8-foot, $^1/_8$-inch glass column packed with 5% DC-11 on HMDS Chromosorb W and a tritium concentric tube detector. Column temperature was 200° ± 0.2° C. The nitrogen flow rate was 65 ml. per minute. Under these conditions, the butoxyethyl esters of the two growth regulators are well resolved from each other. Growth regulators were identified by retention time of their butoxyethyl ethers by gas chromatography.

Experimental Procedures and Results

In an initial experiment, 300-gram samples of fresh peel were heated in a convection-type oven at 130° C. for 4, 6, 8, 10, and 12 hours, respectively. A maximum amount of total residue became available for extraction from the peel of oranges treated with 2,4-D after heating for 10 hours and from the peel of oranges treated with 2,4,5-TP after 12 hours. The acetone powder residue remaining after extraction was rehydrated and heated for 12 hours. Re-extraction of this material produced additional residues of growth regulator. The total residue recovery was the same in all cases (Table I).

This demonstrated conclusively that heat released a fraction of growth regulator not normally extractable from peel with acetone. Under laboratory conditions, 12 hours' heating time at 130° C. appeared to give the optimum release of the heat-labile fraction. These conditions were used for further investigations.

Table I. Effect of Heating Time on 2,4-D and 2,4,5-TP Release from Orange Peel

Heating Time at 130° C., Hours	Growth Regulator, P.P.B.		
	Found in first extract	Recovered from acetone powder	Total
2,4-D-Treated Oranges			
4	15.6	7.5	23.1
6	18.5	5.2	23.7
8	21.0	4.5	25.5
10	23.7	0	23.7
12	23.7	0	23.7
2,4,5-TP-Treated Oranges			
4	11.4	15.1	26.5
6	13.1	11.9	25.0
8	17.6	6.6	24.2
10	19.0	5.5	24.5
12	26.2	0	26.2

The acetone powder remaining after extraction of the peel was rehydrated to prevent charring, heated for 12 hours at 130° C., then analyzed.

Samples of fresh peel were analyzed before and after heating, determining all three fractions. The respective acetone powders remaining after extraction of peel were heat-treated and re-extracted. These extracts were also fractionated to determine free acid, ester acid, and soluble, conjugated acid. These results are shown in Table II.

Free acid, ester acid, and soluble conjugated acid were found in the peel by direct acetone extraction. When the acetone powder from this extraction is heat-treated and reanalyzed, a new fraction, liberated by heat, appears in conjugated form which yields free acid on hydrolysis with acid or alkali by the same conditions under which the extractable conjugate is hydrolyzed (*5*). When the peel is heat-treated prior to extraction no further residue can be recovered from the acetone powder. This provides a method for differentiating between the soluble conjugated residue and the solubilized heat-labile form. A total analysis of heat-treated peel hydrolyzed before separation of fractions, yielded results in good agreement with the total of the individual fractions.

Fractions found in feed samples containing 2,4-D and 2,4,5-TP, respectively,

Table II. Effect of Heat on Release of 2,4-D and 2,4,5-TP Fractions in Orange Peel

	Free Acid, P.P.B.	Ester Acid, P.P.B.	Soluble or Heat-Labile Conjugated Acid, P.P.B.	Total Acid, P.P.B.
2,4-D-treated oranges				
Fresh peel (unheated)	9.3	3.7	7.3	20.3
Acetone powder[a]	0	0	8.9	29.2
Dried peel (heated)	9.6	4.5	13.7	27.8
Acetone powder[a]	0	0	0	27.8
Dried peel (total residue analysis)	...	...	...	27.6
Feed[b]	14	0.3	57	71.3
2,4,5-TP-treated oranges				
Fresh peel (unheated)	9.1	5.8	10.6	25.5
Acetone powder[a]	0	0	9.0	34.5
Dried peel (heated)	10.6	6.4	16.4	33.4
Acetone powder[a]	0	0	0	33.4
Dried peel (total residue analysis)	...	...	...	32.0
Feed[b]	20	0.3	52	72.3

[a] Acetone powder remaining after extraction of the peel was rehydrated to prevent charring and heated 12 hours at 130° C., then analyzed.
[b] Calculated back to fresh weight basis for ease of comparison.

are included in Table II. For ease of comparison, these values are expressed on a fresh weight basis. These data reveal that the total of the conjugated forms in the feed is very much higher than those of the fresh peel. This may be partially explained by the fact that this feed was made from oranges picked when the fruit drop study was terminated 4 months after spraying. It appears that the fruit accumulates much of the growth regulator residue in an insoluble form conjugated with some peel constituent, since the soluble residues formed in the peel of the fruit at the time of harvest could account for but 29 p.p.b. of the 72 p.p.b. present in the feed.

Very low concentrations of these growth regulators have been found in orange juice. One sample of orange juice containing 2,4-D residue was investigated to determine whether heat-labile forms of 2,4-D were present.

A sample of orange juice prepared by reaming in an electric juicer was extracted with acetone. The total soluble residue was found to be 2.9 p.p.b. A second portion of the same juice was also extracted with acetone. The clear serum (following removal of acetone) and the insoluble juice solids were heated at 130° C. After 24 hours, the serum yielded 3.0 p.p.b. of 2,4-D residue (the serum was heated for 24 hours, so that it became concentrated to a sirup and was at approximate equilibrium with oven temperature). The insoluble juice solids (heated for 12 hours) on extraction yielded 1.0 p.p.b. of (original sample weight basis) heat-labile 2,4-D. Since most of the insoluble solids in the juice of reamed fruit normally are not present in commercial juice, this small amount of heat-labile residue is of negligible practical significance. No heat-labile residue occurs in the serum.

When peel is heated there is a time lapse before the peel is dry and at equilibrium with oven temperature, so that the peel is heated in the presence of moisture for part of the total heat treatment. The effect of moisture on the heat-releasing mechanism was studied. Samples of orange peel containing 2,4-D were lyophilized and heated at 130° C. for various periods of time up to 4 hours. Longer heating was not possible because the fluffy material tended to char. Extractable residue increased steadily with time of heating. After 4 hours, the extractable residue was 12.2 p.p.b. A control sample of the same material in fresh condition, heated for 12 hours, contained 12.0 p.p.b. Moisture is not necessary for the release of this fraction. A dehydration type of reaction is probable.

Earlier experiments reported by Meagher (*5*) showed that "2,4-D and 2,4,5-TP peel" acetone powders did not yield more extractable residue when heated for 1 hour in 0.5*N* KOH or 0.5*N* HCl. Papain and pepsin digestion also were ineffective for releasing additional residue.

More rigorous conditions were tried in an attempt to attain release of residues from peel by heating samples for 5 hours at 100° C. in both 0.5*N* and 5*N* KOH or H_2SO_4. Only a small portion of the heat-labile fractions was released. No special benefit was achieved by using the more concentrated reagents. The results of acid and base treatments were similar. The heat-labile fractions are very resistant to hydrolysis in situ, even though, when solubilized, hydrolysis can be carried out with relative ease. Possibly, heat alone was responsible for the small releases that were achieved.

Acetone as an extractant has been found to yield consistent results. Ethyl ether, as reported by Yip (*6*), for extraction of 2,4-D herbicides, and 2-propanol, used by Hagin and Linscott (*3*) for extraction of 2,4-D, were also tried. A direct extraction of peel was made, then the insoluble residue was hydrated, heated at 130° C. for 12 hours, and re-extracted with the same two solvents. The results, when compared with acetone extraction, were 30 to 40% low. Although reported to be satisfactory for many other plant materials, these solvents apparently are unsuited for extracting these types of growth regulators from oranges.

Initial exploratory experiments have been conducted on a semiquantitative scale to determine the form of the heat-labile fraction in the peel and to characterize the soluble forms of the conjugates. A small amount of pectic acid was extracted with 0.01*N* KOH from an acetone powder containing heat-labile 2,4-D. The pectic acid was precipitated with HCl and collected by filtration. After heating in the oven in the usual manner, mixed with an acetone powder free of growth regulators to give bulk, a 2,4-D conjugate was released. Very roughly the 2,4-D conjugate could account for 25 p.p.b. as heat-labile residue in the peel.

Samples of hydrated feed containing 2,4-D and 2,4,5-TP, respectively, were extracted with acetone. Aqueous extracts after evaporation of acetone were passed through ion exchange columns of Dowex 50 (H^+) and Dowex 1 (OH^-) resins. The conjugated forms of the growth regulator were recovered in the effluent liquors, indicating that neither of the conjugates contains acidic or basic groups. The neutral effluents were subjected to successive 24-hour continuous extractions with petroleum ether, ethyl ether, and ethyl acetate, respectively. After evaporation of the solvent, hydrolysis in 0.5*N* KOH and analysis revealed that growth regulators were present in all three solvent fractions. These latter results were qualitative in nature. No growth regulators remained in the aqueous phase.

Discussion

Crosby (*2*), investigating 2,4-D metabolites in bean plants, has reported a water-soluble, ether-insoluble, readily hydrolyzable substance containing 2,4-D. The ether extract of the bean plant contained 2,4-D as the free acid. Earlier work of Andreae and Good (*1*) indicated that a conjugated form, 2,4-dichlorophenoxyacetylaspartic acid, is formed in bean plants. Crosby (*2*) eliminated this as the probable conjugate because this compound would be ether-soluble. Klämbt (*4*), on the other hand, has suggested that 2,4-D may be bound as a 2,4-dichlorophenoxyacetylglucoside. This is ether-insoluble and reported to be soluble in ethyl acetate. The apparent association of the heat-labile fraction with pectin indicates that a conjugated form of the growth regulator with some carbohydrate is plausible. 2,4,5-TP could undergo similar reactions.

The nature of the materials extracted in the three solvent fractions is purely speculative. All acidic and basic groups were removed by ion exchange in the columns. Any amino acid conjugates, were they present, would be removed. The extremely low concentrations of the conjugates in orange peel make actual isolation of pure material a formidable task.

The main point of emphasis in this report is that halogenated phenoxy alkanoic acids may exist in many unidentified forms in plant material. Simple analyses for free acid content can give rise to low residue concentrations. The heat-labile fraction in oranges, as well as the soluble conjugate, constitutes an important part of these growth regulator residues. Investigators using related compounds on other plants should realize that similar conjugates are likely to be present in these species as well.

Acknowledgment

The author thanks B. G. Shively for his valuable technical assistance in obtaining many of the data.

Literature Cited

(1) Andreae, W. A., Good, N. E., *Plant Physiol.* **32,** 566 (1957).
(2) Crosby, D. G., J. AGR. FOOD CHEM. **12,** 2 (1964).
(3) Hagin, R. D., Linscott, D. L., *Ibid.*, **13,** 123 (1965).
(4) Klämbt, H., *Planta* **57,** 391 (1962).
(5) Meagher, W. R., J. AGR. FOOD CHEM. **14,** 374 (1966).
(6) Yip, G., *J. Assoc. Offic. Agr. Chemists* **47,** 343 (1964).

Received for review June 16, 1966. Accepted August 31, 1966. Florida Agricultural Experiment Stations Series No. 2419.