Spray Residues of 2,4-D and 2,4,5-TP in 'Pineapple' Orange Peel

Rudolph Hendrickson and W. R. Meagher[1]

Electron-capture gas chromatography was employed to investigate the metabolites of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-(2,4,5-trichlorophenoxy) propionic acid (2,4,5-TP) in 'Pineapple' orange peel. Maximum residues of 2,4-D and 2,4,5-TP (200 p.p.b.) were recovered from the peel approximately 5 weeks after field application of a dilute spray (20 p.p.m.). A heat-labile fraction, not extracted with acetone, became the predominant metabolite in a matter of weeks and showed the greatest persistence. The procedure for recovering total 2,4-D and 2,4,5-TP from citrus peel is also described.

In Florida, dilute sprays (20 p.p.m.) of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-(2,4,5-trichlorophenoxy) propionic acid (2,4,5-TP) applied on 'Pineapple' orange trees have effectively controlled excessive preharvest fruit drop (Phillips and Meagher, 1966). Only 2,4-D is presently registered for this use, although 2,4,5-TP is as effective without causing damage to the spring flush of foliage that occasionally occurred after 2,4-D applications.

This study elucidates the metabolic fate of these growth regulators over a 13-week period following its spray application on 'Pineapple' orange trees. Residues of 2,4-D and 2,4,5-TP were determined as butoxyethyl esters by electron-capture gas chromatography, using the procedure of Meagher (1966a) and a later modification (Meagher, 1966b).

In addition, the direct procedure by Meagher (1966b) for analyzing total 2,4-D or 2,4,5-TP in citrus is described.

METHODS AND MATERIALS

Spray Composition. Each spray was diluted to contain 20 p.p.m. of the free acid of isopropyl 2,4-D (Weedicide, Thompson Chemical Co.), propyleneglycol butyl ether ester of 2,4,5-TP (Kuron, Dow Chemical Co.) and triethanolamine salt of 2,4,5-TP. Since the latter was no longer available commercially, it was synthesized and formulated with X77 sticker spreader (Chevron Chemical Co.). The triethanolamine salt was included in these trials because Sites (1954) had effectively used it on citrus for fruit drop control.

Application and Experimental Design. Each formulation was thoroughly applied to the foliage of four 'Pineapple' orange trees in the fall (October 26) with a conventional high-pressure sprayer.

Sampling. Each sample comprised 16 fruit (four fruit per tree) picked daily for the first 15 days, and then weekly. The unwashed fruit was hand-juiced by electric reamer and the remaining peel was coarsely chopped before a 500-gram composite sample was taken for analysis. Peel samples were stored occasionally (although not during the first 6-week period) in polyethylene bags at −40° C. prior to analysis.

Analysis. The procedure for determining the total residue of 2,4-D in citrus peel, without separation of specific fractions, is as follows: Place 500 grams of composite peel in a 1-liter beaker and heat for 24 hours in an oven held at 104-5° C. Stir the sample every 2 hours, when possible, to assure a maximum time interval of high temperature and low moisture content. Remove the dried peel (moderately brown, but not charred) from the oven; add 300 ml. of water and let stand for 24 hours. Blend the rehydrated peel with 600 ml. of acetone in a 1-gallon Waring disintegrator at maximum speed for 2 minutes or at medium speed for 3 minutes (if mixture gels, add more acetone or use a shorter blending time). Filter the slurry through an 8-inch Büchner funnel with Whatman No. 50 filter paper; when filtration is almost complete, quickly flood the Büchner funnel with 300 ml. of acetone and aspirate dry. Add an additional 200 ml. of acetone to the Büchner funnel if the filter-cake is not powdery-dry. Once the filter-cake is fluffy dry, it is discarded. The acetone is removed from the combined filtrate in a rotary flash evaporator at a water bath temperature not exceeding 40° C. Neutralize the remaining liquid with 1*N* KOH, then add more KOH pellets (or as a concentrated solution) to make the extract 0.5*N* KOH. Heat the extract in a 1-liter round-bottomed flask on a water bath at 90° to 98° C., and hold for 15 minutes with the neck of the flask restricted by an air condenser or short-stemmed funnel. Cool and acidify the 0.5*N* KOH solution, adding an equivalent volume of 0.7*N* HCl. Transfer the solution to a 1-liter separatory funnel and extract the acidified solution three times with 100-ml. portions of hexane, avoiding emulsification by mechanically tumbling each extraction for 3 minutes at slow speed. Discard the water phase. The combined hexane extracts are gently extracted with 100 ml. of 0.2*M* K_2HPO_4 and extracted twice more with 50-ml.

Institute of Food and Agricultural Sciences, University of Florida Citrus Experiment Station, Lake Alfred, Fla. 33850

[1] Deceased

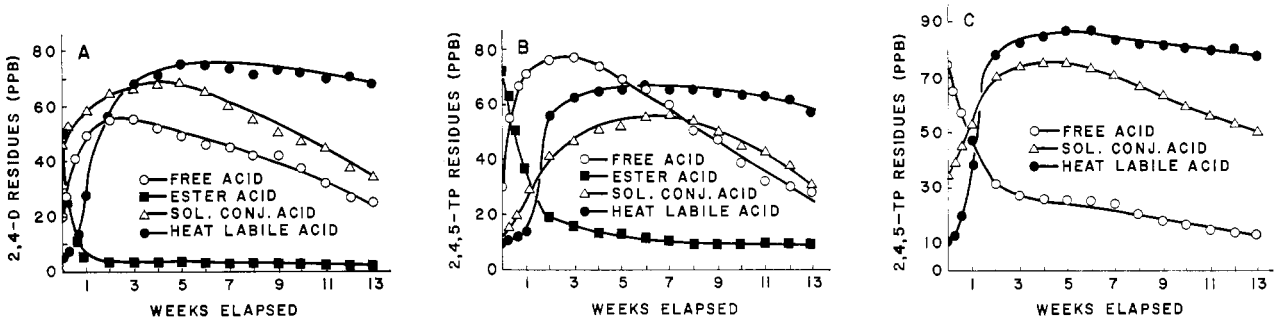

Figure 1. Parts per billion of metabolites found in "Pineapple" orange peel

A. After applying the isopropyl ester of 2,4-D
B. After applying the butyl ether ester of 2,4,5-TP
C. After applying the triethanolamine salt of 2,4,5-TP

portions, thereby partitioning the free acid into the aqueous phase. Wash the aqueous phase once with 25 ml. of chloroform, discard the chloroform, and acidify with 25 ml. of 2*N* H_2SO_4. Extract the aqueous phase once with 25 ml. and twice more with 10 ml. of chloroform. The combined chloroform extracts are then carefully evaporated to dryness in a 50-ml. Erlenmeyer flask. A combination of air movement and gentle heat (top of warm steam bath) is suggested initially, and then only a mild hood draft as the last traces of chloroform are removed. The esterification procedure and gas chromatographic technique from this point have been described by Meagher (1966a).

The total residue of 2,4,5-TP in citrus peel is determined by the same procedure, but an additional alkaline hydrolysis time (30 minutes *vs.* 15 minutes) is required.

RESULTS AND DISCUSSION

In this study, 2,4-D and 2,4,5-TP were recovered as four fractions: free acid, ester acid (hexane-soluble), conjugated acid (water-soluble, hexane-insoluble), and a heat-labile conjugated acid (heat-released). Previous publications by Meagher (1966a, 1966b) described the technique for isolating fractions of the total 2,4-D and 2,4,5-TP residue from citrus peel. A sequence of extraction and heating divided the total residue into four respective fractions that are converted to the free acid, esterified, and then quantitated by gas chromatography, utilizing an electron-capture detector. Similar fractions were found by previous investigators in plants after use of 2,4-D or 2,4,5-TP. Erickson *et al.* (1963) provided evidence that all of the isopropyl ester of 2,4-D in the cells of treated lemons was hydrolyzed and that ester-like residue was synthesized in vivo. Later, Crosby (1964), investigating 2,4-D metabolites in bean plants, reported the conversion of absorbed 2,4-D into a water-soluble, ether-insoluble derivative which was readily hydrolyzed to highly active 2,4-D. A heat-labile fraction of 2,4-D and 2,4,5-TP was found in citrus by Meagher (1966b), and these phenoxy compounds were suggested as being conjugated with the pectin of citrus.

The respective concentrations of these residue fractions in orange peel during the 13-week period following spray application are graphically presented in Figure 1. These data suggest that the original ester or free acid fraction, in the case of the amine salt, was rapidly metabolized and apparently transformed into the free acid. Free acid was then actively metabolized to soluble conjugated forms. Concomitantly, fractions of residue were transfixed to an insoluble conjugated form. After 3 to 5 weeks, the concentration of the heat-labile fraction was stabilized at a high level, while the concentrations of free and soluble conjugated fractions began a gradual decrease. Ultimately, the insoluble conjugated residue appeared to become the major part of the total growth regulator residue. Dried citrus pulp processed from sprayed orange peel contained 60% of the total residue in the insoluble conjugated form 13 weeks after being sprayed. Concurrent experiments in which 'Pineapple' oranges were dipped in phenoxy spray formulations indicated that similar residue changes occurred, but at a more accelerated rate.

Seeds and juice were analyzed periodically. Total phenoxy residue in seeds showed little change with time, and consistently contained less than 1.0 p.p.b., as did the juice, although in other similar studies juice contained up to 3 p.p.b. These values were at the lower detection limit and were considered insignificant.

The total 2,4-D or 2,4,5-TP residue in the peel of the fruit one day after spray application in these experiments averaged about 120 p.p.b., as shown in Figure 2. Total esters or salt residue was noted as increasing to 190 to 200 p.p.b. in 2 to 5 weeks, then declining again to 125 p.p.b. by the end of 13 weeks.

In comparison, an earlier 2,4-D investigation by Erickson and Hield (1962) with a 20-p.p.m. isopropyl

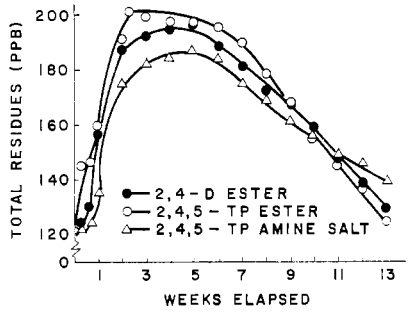

Figure 2. Parts per billion of total residue in 'Pineapple' orange peel at weekly time intervals after spray application of the respective growth regulator

ester formulation sprayed on 'Washington Navel' oranges brought about 0.1 p.p.m. total residue in the fresh fruit one day after application. Seven days later, only two-thirds of this concentration was found, the major portion being an acid fraction and the remainder an ester fraction. Low concentration of 2,4-D in the respective fractions precluded any reliable estimate of ester hydrolysis by relatively insensitive microcoulometric gas analyses. However, in lemon dipping experiments, using much higher concentrations of 2,4-D (500 p.p.m.), Erickson and Hield found 90% of the 2,4-D was hydrolyzed in two days. Results obtained in the present investigation (Figure 1) suggest a lower rate of hydrolysis. The more sensitive analytical procedure, the additional fraction of 2,4-D liberated, and the interval after spraying led to a much greater residue in Florida 'Pineapple' oranges than in 'Washington Navel' oranges of the Erickson-Hield study.

Finally, the slow disappearance of the heat-labile, 2,4-D residue in citrus would implicate this less-known conjugate as the more persistent form. In view of the possible association of this residue with pectin and the occurrence of pectin in perhaps all plant tissues, other investigators should be challenged by its implication.

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Received for review September 16, 1968. Accepted January 9, 1969. Florida Agricultural Experiment Stations Journal Series No. 3061.