

## Diphenyl Absorption by Honey Tangerines: The Effects of Washing and Waxing and Time and Temperature of Storage

Steven Nagy\* and Wilfred F. Wardowski

Temperature, storage time, fruit preparation, number of diphenyl pads packed per carton, and harvest time were evaluated to determine their influence on the extent of diphenyl absorption by Honey tangerines. Washed and waxed fruit stored for 4 weeks at 4 and 21 °C absorbed about 20–25 and 50–65 ppm of diphenyl, respectively, when packed with one diphenyl pad and about 25–35 and 70–85 ppm, respectively, with two diphenyl pads. Unwashed fruit stored under similar conditions absorbed about 10–15 and 45–60 ppm, respectively, with two pads. Storage of fruit packed with two diphenyl pads afforded the greatest protection against decay, but temperature and storage time must be carefully controlled because of the higher amounts of diphenyl absorbed. The average diphenyl contents of fruit stored at 21 °C with two diphenyl pads were below the legal tolerance limit (110 ppm) established by the United States but not necessarily below the European and Japanese tolerance of 70 ppm.

Diphenyl is a fungistat used to control decay of citrus fruits. Since first reported by Tomkins (1935), diphenyl has proved effective in controlling stem-end rot fungi and sporulation of green mold. In commercial practice, pads of kraft paper impregnated with diphenyl (2.2 g per 29 by 43 cm pad) are inserted into  $\frac{4}{5}$  bushel (28.2 L) cartons during packing of citrus fruit. Diphenyl, which volatilizes from the pads, inhibits fungal vegetative growth and spore formation (Ramsey et al., 1944) and, also, is absorbed by the fruit. The legal diphenyl residue tolerance for citrus fruits marketed within the United States, Canada, and Sweden is 110 ppm, whereas 70 ppm is the legal tolerance for citrus fruit sold in Japan and countries belonging to the European Economic Community (Nagy and Wardowski, 1980).

Honey tangerine (previously named Murcott) is the second most abundant tangerine produced in Florida. During the 1978–1979 season, Florida produced about 1.77 million boxes (1 box equals 43.1 kg or 95 lb of fruit; State of Florida, 1974) of Honey tangerines and, of this quantity, sold 1.01 million boxes as fresh fruit for 8.9 million dollars (Florida Crop and Livestock Reporting Service, 1979). Although minor by comparison with oranges and grapefruit, export of Honey tangerines has increased in recent years: from 0.09 million boxes during the 1977–1978 season to over 0.18 million boxes during the 1979–1980 season (Brady, 1980).

Previous investigations on the use of diphenyl showed that the amount of diphenyl absorbed by citrus fruits varied with storage conditions (Hayward and Edwards, 1963; Hayward et al., 1966; Norman et al., 1971; Wardowski et al., 1979), packaging methods (Rygg et al., 1964; Hayward et al., 1965), variety of citrus (Hayward and Edwards, 1963, 1964; Norman et al., 1971), and the physiological state of the fruit (Norman et al., 1971; McCornack, 1976). The present investigation was prompted by previous studies from our laboratory (Hayward and Edwards, 1963; Hayward et al., 1965) which showed that tangerines absorbed more diphenyl than oranges, grapefruit, lemons, and limes under similar storage conditions. Because of the commercial importance of Honey tangerine, a study was undertaken to evaluate the effects of temperature, length of storage, number of diphenyl pads per carton, and the

condition of the fruit (washed and waxed vs. unwashed) on the decay percentage and the amount of diphenyl absorbed by these fruit. Unwashed fruit were included to determine if the fruit's natural wax barrier exerted any effect on the amount of diphenyl absorbed.

### EXPERIMENTAL SECTION

**Fruit.** Mature Honey tangerines were harvested from trees growing at the Agricultural Research and Education Center, Lake Alfred, on Feb 28 and March 14, 1979 (1978–1979 citrus season), and on Jan 28 and Feb 25, 1980 (1979–1980 citrus season).

**Washing, Packing, and Storing.** Fruit were randomized and divided; half of each sample was washed and waxed with a solvent-type wax, whereas the other half remained unwashed and unwaxed. All fruit (60 fruit per treatment) were packed in standard  $\frac{4}{5}$  bushel cartons in which the ventilation holes were taped closed to simulate conditions in solid loads. For each experiment, random-size fruit packed with zero, one, or two diphenyl pads per carton were placed at 4 °C (40 °F) and 21 °C (70 °F). For the cartons containing two pads, the diphenyl pads were placed at the bottom of the carton and over the top layer of fruit, whereas for the one-pad-containing cartons, the pad was only placed at the bottom.

**Sampling and Analysis.** Samples of 10 fruit were removed from each carton at 2-week intervals according to the procedure of Hayashi (1971). The fruit were cut into several pieces, placed in a Waring blender, and macerated to a homogeneous puree in ~2 min. One hundred grams of homogenized sample was transferred to a 3-L round-bottom flask and 300 mL of water was added. To this mixture, 1 mL of concentrated sulfuric acid, 0.5 mL of silicone antifoam, and ~10 boiling stones were added. Diphenyl was distilled and captured in cyclohexane with a liquid-liquid extractor according to the method of Newhall et al. (1954). Samples of diphenyl in cyclohexane were analyzed by gas-liquid chromatography (GLC) with either a Hewlett-Packard Model 5730A or a Hewlett-Packard Model 5880A gas chromatograph equipped with a flame ionization detector. Diphenyl was resolved from other components of the extract with a column (1.83 m in length and 2 mm i.d.) packed with 5% Carbowax 20 M on 100–200-mesh Supelcoport (Supelco, Inc., Bellefonte, PA). The injection port and detector were at 200 °C, and the nitrogen flow was 60 mL/min. Samples of 2  $\mu$ L were injected on-column at 120 °C, programmed at 4 °C/min up to 190 °C, and, finally, held isothermally at 190 °C for 2 min. By

Florida Department of Citrus (S.N.) and Cooperative Extension Service (W.F.W.), Agricultural Research and Education Center, Lake Alfred, Florida 33850.

Table I. Storage of Honey Tangerines at 4 °C: Effects of Fruit Treatment, Number of Diphenyl Pads, Harvest Period, and Weeks of Storage on Decay Percentage and Diphenyl Content<sup>a</sup>

pads	washed and waxed fruit				unwashed fruit			
	2 weeks		4 weeks		2 weeks		4 weeks	
	% decay	diphenyl, ppm	% decay	diphenyl, ppm	% decay	diphenyl, ppm	% decay	diphenyl, ppm
Harvest A (1/28/80)								
0	0.7 ± 1.2 <sup>a</sup>		12.3 ± 10.8 <sup>a</sup>		2.7 ± 2.3 <sup>a</sup>		3.4 ± 5.7 <sup>a</sup>	
1	0 <sup>a</sup>	20.2 ± 5.2 <sup>a</sup>	0.2 ± 0.2 <sup>a</sup>	25.3 ± 5.7 <sup>a</sup>	2.0 ± 0 <sup>a</sup>	10.3 ± 4.6 <sup>a</sup>	0.4 ± 0.4 <sup>a</sup>	13.8 ± 2.4 <sup>a</sup>
2	1.3 ± 1.2 <sup>a</sup>	21.9 ± 3.8 <sup>a</sup>	0.3 ± 0.3 <sup>a</sup>	34.3 ± 2.2 <sup>b</sup>	0 <sup>a</sup>	14.9 ± 4.2 <sup>a</sup>	0.1 ± 0.2 <sup>a</sup>	22.6 ± 2.8 <sup>b</sup>
Harvest B (2/25/80)								
0	0.7 ± 1.2 <sup>a</sup>		6.0 ± 5.3 <sup>a</sup>		0 <sup>a</sup>		1.0 ± 1.7 <sup>a</sup>	
1	0 <sup>a</sup>	17.7 ± 1.9 <sup>a</sup>	6.0 ± 4.4 <sup>a</sup>	21.7 ± 1.6 <sup>a</sup>	0 <sup>a</sup>	8.3 ± 2.4 <sup>a</sup>	5.0 ± 5.0 <sup>a</sup>	12.9 ± 0.8 <sup>a</sup>
2	0 <sup>a</sup>	20.1 ± 3.7 <sup>a</sup>	0 <sup>a</sup>	26.4 ± 2.0 <sup>b</sup>	0 <sup>a</sup>	9.1 ± 1.1 <sup>a</sup>	4.3 ± 4.0 <sup>a</sup>	18.7 ± 1.9 <sup>b</sup>

<sup>a</sup> Values represent the mean ± standard deviation for three replications. Means in the same column followed by the same letter are not different at the 5% level of significance (Duncan's multiple range test).

use of a Hewlett-Packard Model 3385A automation system or 5880A Level Four system, concentrations of unknown diphenyl samples were determined by comparison to a standard diphenyl concentration plot.

Samples collected and analyzed during the 1978–1979 season were run in duplicate and the results averaged for the Feb 28 and March 24 harvests. This first study was intended to demonstrate patterns or show relationships among weeks of storage, number of pads per carton, and the condition of the fruit. To this end, fruit was stored for 10 weeks at 4 °C and analyzed for diphenyl at biweekly intervals. Samples collected and analyzed during the 1979–1980 season were run in triplicate and were stored for 4 weeks at both 4 and 21 °C. With these fruit, not only was diphenyl absorption measured under various storage conditions but also the percentage of decayed fruit for each treatment was determined.

## RESULTS AND DISCUSSION

The amounts of diphenyl residue from samples stored for 10 weeks at 4 °C and analyzed at biweekly intervals are shown in Figure 1. Fruit that were washed and waxed, and packed with either one or two diphenyl pads, showed an increase in diphenyl residue with time. After 10 weeks, fruit packed with one pad showed an average diphenyl residue content of 49 ppm (mg/kg of fruit), whereas fruit packed with two pads showed an average residue of 66 ppm. Unwashed fruit (also Figure 1) showed similar patterns of increased diphenyl residues with time. However, the amount of diphenyl absorbed by unwashed fruit was noticeably lower (~50% less) than the washed and waxed fruit. After 10 weeks, unwashed fruit packed with one and two pads contained an average of 26 and 30 ppm of diphenyl, respectively. As expected, whether fruit were washed or unwashed, higher diphenyl residue levels were found in fruit packed with two pads than when packed with only one pad. With information gathered from the 1978–1979 Honey tangerine storage experiments, a more extensive study on the effects of storage conditions on the 1979–1980 crop was undertaken.

Table I lists the amounts of diphenyl absorbed and the percentage of decay by fruit harvested on Jan 28 (harvest A) and Feb 25, 1980 (harvest B), and subsequently stored at 4 °C.

**Harvest A at 4 °C.** Washed and waxed fruit packed with either one or two pads showed minimal decay of ~1% or less after 2 and 4 weeks of storage. After 4 weeks, fruit packed with no diphenyl pads showed an average decay of ~12%; however, no significant difference (5% level) was determined between fruit packed with or without diphenyl pads by Duncan's multiple range test. The highest average amount of diphenyl absorbed by these washed and waxed

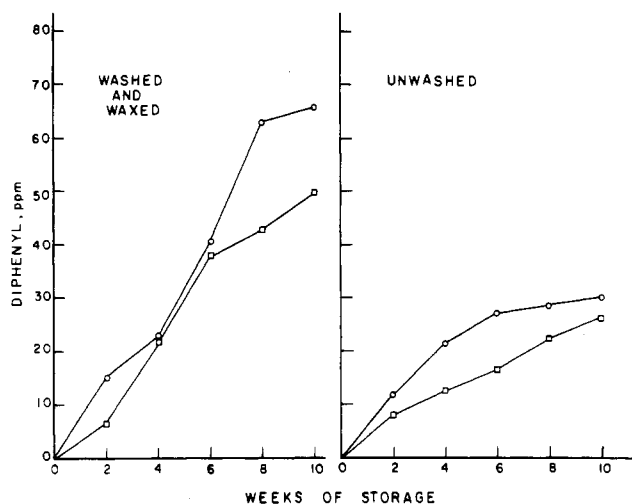


Figure 1. Uptake of diphenyl by washed and waxed and by unwashed Honey tangerines packed in cartons containing one pad (□) or two pads (○) during a 10-week storage period at 4 °C.

fruit was ~34 ppm after 4 weeks. Whereas no significant difference was found after 2 weeks in the amounts of absorbed diphenyl by fruit packed with one or two pads, a significant difference was observed after 4 weeks. The decay percentages of unwashed fruit packed with or without diphenyl pads were statistically nonsignificant (Duncan's test, Table I). Occasionally, the average decay percentage of fruit in some cartons after 2 weeks was slightly higher than the decay percentage after 4 weeks. This is due to decay variability. Although every effort was made to cull the bad fruit carefully prior to packaging, some fruit predisposed to decay undoubtedly passed visual inspection. Therefore, cartons in which these fruit were packaged would tend to have higher average decay percentages than cartons containing all sound fruit. Sampling was done without replacement, i.e., the replicated cartons for the 2-week period were different from the replicated cartons of the 4-week period. Unwashed fruit absorbed statistically lower amounts of diphenyl than washed and waxed fruit. A significant difference in diphenyl absorption was noted after 4 weeks with fruit packed with one and two pads but not after 2 weeks (Table I).

**Harvest B at 4 °C.** Fruit harvested on Feb 25 showed diphenyl absorption patterns and decay percentages similar to those for fruit of harvest A. Fruit packed with two pads generally had the lowest average decay percentages. However, comparative examination of decay by Duncan's test (Table I) showed no statistical difference by fruit packed with or without diphenyl pads. Washed and unwashed fruit stored for 4 weeks with two pads absorbed

about 5 and 6 ppm more diphenyl, respectively, than similarly treated fruit packed with one pad (significant by Duncan's test). As noted previously, fruit left unwashed absorbed less diphenyl than washed and waxed fruit.

**Harvest A at 21 °C (Table II).** The effects of a higher storage temperature on the percentage of fruit decay were readily apparent. Washed and waxed fruit stored for 2 weeks with no diphenyl pad showed a significantly higher decay than fruit packed with one or two pads. Also, fruit stored for 4 weeks with no pad showed a significantly higher decay when contrasted to the two-pad cartons but not the one-pad cartons. At 2 and 4 weeks, washed and waxed fruit packed with two pads absorbed about 30 and 66% more diphenyl, respectively, than fruit packed with one pad. Unwashed fruit showed lower average decay percentages when packed with diphenyl pads. The decay percentages for the zero-, one-, and two-pad cartons were not significantly different at 2 weeks, but at 4 weeks the two-pad cartons differed significantly from the zero- and one-pad cartons. Unwashed fruit packed with two pads and stored 2 weeks contained ~74% more diphenyl than the one-pad carton, and when stored for 4 weeks it contained ~47% more diphenyl.

**Harvest B at 21 °C.** Decay percentages and the amounts of diphenyl absorbed followed patterns similar to those of harvest A (21 °C). Washed and waxed fruit after 4-weeks storage (zero, one, or two pads) showed lower average decay percentages than unwashed fruit (zero, one, or two pads) after a comparable storage period. Comparison of 4 week stored fruit (washed and waxed vs. unwashed) by the *t* statistic for two means confirmed a significant difference (5% level) for the zero- and one-pad-containing fruit cartons but not for the two-pad cartons. Washed and waxed fruit packed with one or two pads absorbed statistically similar amounts of diphenyl (Duncan's test); however, unwashed fruit packed with one or two pads absorbed significantly different amounts of diphenyl.

After 4 weeks storage at either 4 or 21 °C, washed and unwashed fruit showed average diphenyl residues below the legal tolerance level of 110 ppm for fruit marketed within the United States. Fruit exported to Japan and countries of the European Economic Community where the tolerance is 70 ppm should be transported at cool temperatures (McCornack et al., 1976) because of the lower decay percentage and the amount of diphenyl absorbed. Although the effects of time, temperature, and number of pads on the extent of diphenyl absorption by unwashed fruit were evaluated, the marketing of unwashed fruit in commercial channels is not recommended. The surface of unwashed fruit usually carries an assortment of dead and living fungi and bacteria (Jahn et al., 1970; Albrigo and Carter, 1977).

Washing and brushing partly or entirely remove the natural wax coating of citrus fruits (ISO, 1978). Studies at 4 °C (Figure 1; Table I) suggest that the natural wax coating of unwashed Honey tangerine reduces the amount of absorbed diphenyl when compared to the amount absorbed by washed and waxed fruit. A comparative examination of diphenyl absorption by washed and waxed vs. unwashed fruit is shown in Table III. Unwashed fruit stored at 4 °C and packed with one or two pads all absorbed statistically lower amounts of diphenyl when compared to washed and waxed fruit. Storage of fruit, however, at 21 °C did not clearly show this difference. Unwashed fruit packed with one pad showed statistical differences in three (harvest A, 2 weeks; harvest B, 2 weeks; harvest B, 4 weeks) out of four treatments. Fruit packed

Table II. Storage of Honey Tangerines at 21 °C: Effects of Fruit Treatment, Number of Diphenyl Pads, Harvest Period, and Weeks of Storage on Decay Percentage and Diphenyl Content<sup>a</sup>

pads	washed and waxed fruit						unwashed fruit					
	2 weeks		4 weeks		4 weeks		2 weeks		4 weeks		4 weeks	
	% decay	diphenyl, ppm	% decay	diphenyl, ppm	% decay	diphenyl, ppm	% decay	diphenyl, ppm	% decay	diphenyl, ppm	% decay	diphenyl, ppm
0	32.7 ± 2.3 <sup>a</sup>		42.0 ± 10.4 <sup>a</sup>		Harvest A (1/28/80)	24.7 ± 11.7 <sup>a</sup>		71.3 ± 5.5 <sup>a</sup>		58.4 ± 6.8 <sup>a</sup>		85.7 ± 12.6 <sup>b</sup>
1	18.0 ± 2.0 <sup>b</sup>	59.1 ± 9.9 <sup>a</sup>	25.0 ± 17.4 <sup>a,b</sup>	51.6 ± 8.9 <sup>a</sup>		19.7 ± 13.4 <sup>a</sup>	40.3 ± 9.3 <sup>a</sup>	55.0 ± 13.9 <sup>a</sup>				
2	16.0 ± 4.0 <sup>b</sup>	76.9 ± 7.7 <sup>b</sup>	13.7 ± 1.2 <sup>b</sup>	85.7 ± 11.2 <sup>b</sup>		7.7 ± 2.9 <sup>a</sup>	70.0 ± 12.8 <sup>b</sup>	20.7 ± 8.6 <sup>b</sup>				
0	16.0 ± 7.2 <sup>a</sup>		47.7 ± 6.0 <sup>a</sup>		Harvest B (2/25/80)	17.3 ± 4.2 <sup>a</sup>		74.3 ± 10.8 <sup>a</sup>				
1	13.3 ± 1.6 <sup>a</sup>	63.2 ± 6.5 <sup>a</sup>	18.3 ± 2.5 <sup>b</sup>	65.4 ± 5.8 <sup>a</sup>		12.7 ± 5.0 <sup>a</sup>	35.8 ± 3.0 <sup>a</sup>	50.3 ± 11.6 <sup>a,b</sup>				47.3 ± 5.8 <sup>a</sup>
2	16.0 ± 10.0 <sup>a</sup>	61.2 ± 10.9 <sup>a</sup>	18.3 ± 7.0 <sup>b</sup>	72.2 ± 3.8 <sup>a</sup>		10.0 ± 2.0 <sup>a</sup>	62.6 ± 8.3 <sup>b</sup>	31.0 ± 26.9 <sup>b</sup>				70.1 ± 6.5 <sup>b</sup>

<sup>a</sup> Means in the same column followed by the same letter are not different at the 5% level of significance (Duncan's multiple range test).

Table III. Comparative Examination of Diphenyl Absorption by Washed and Waxed vs. Unwashed Honey Tangerines Using the *t* Statistic for Two Means

harvest	storage time, weeks	no. of diphenyl pads	level of significance, %, for storage at	
			4 °C	21 °C
A	2	1	0.5	0.5
A	2	2	1.0	NS <sup>a</sup>
A	4	1	0.5	NS
A	4	2	0.5	NS
B	2	1	0.5	0.5
B	2	2	0.5	NS
B	4	1	0.5	0.5
B	4	2	0.5	NS

<sup>a</sup> NS = nonsignificant.

with two pads, between washed and unwashed, showed no significant differences in diphenyl absorption levels at 2 and 4 weeks. Apparently the diphenyl vapor pressure from two pads at 21 °C was high enough to overcome the barrier protection of the natural wax coating. This premise appears valid in light of past results by Hayward and Edwards (1964), who showed that waxed and unwaxed oranges packed with two pads absorbed similar amounts of diphenyl when stored at 21 °C.

This study investigated the extent of diphenyl absorption on two tangerine harvests because previous investigations by Rajzman (1965) and Wardowski et al. (1979) indicated that less mature fruit absorbed more diphenyl than more mature fruit. Lemons and oranges with green peel color, for example, absorb more diphenyl than fully colored fruit (Norman et al., 1969, 1971). The results in Tables I and II show with some minor exceptions that fruit harvested in January absorbed higher average amounts of diphenyl than fruit of the February harvest. However, statistical evaluation of the data was inclusive. Further studies on other tangerine cultivars are required to verify the influence of maturity on diphenyl absorption.

Honey tangerines should be stored and shipped at cool temperatures (~4-10 °C) because of the decay rate and the extent of diphenyl absorption at the higher temperatures. Storage of fruit with two diphenyl pads affords the greatest protection against decay but transit temperature and storage time must be carefully monitored because the

fruit is exposed to a greater vapor concentration of diphenyl.

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Received for review November 10, 1980. Accepted March 12, 1981. Florida Agricultural Experiment Stations Journal Series No. 2757.

## Metabolism of 2,5-Dichloro-4-hydroxyphenoxyacetic Acid in Plants

Arbor D. Drinkwine<sup>1</sup> and James R. Fleeker\*

The 2,4-dichlorophenoxyacetic acid (2,4-D) metabolite, 2,5-dichloro-4-hydroxyphenoxy[1-<sup>14</sup>C]acetic acid ([*acetic-1-<sup>14</sup>C]-4-OH-2,5-D) was prepared. The labeled compound was taken up by red currant and carrot shoots and by the roots of intact bean, wheat (durum), and corn plants. After 48 h the tissue was freeze-dried and extracted. From 19 to 32% of the label remained in the residual plant material. A small portion of the radioactivity, 1-10%, was recovered as [<sup>14</sup>C]carbon dioxide. From 9 to 31% of the [<sup>14</sup>C]-4-OH-2,5-D was found unchanged and 15-50% was recovered after treatment of the extracts with emulsin. A large portion of the radioactivity, 29-40%, was highly polar material and not identified. The chromatograms of the plant extracts suggested that the metabolic products were similar.*

The metabolism of 2,4-dichlorophenoxyacetic acid (2,4-D) in plants has been studied by many laboratories

Department of Biochemistry, North Dakota State University, Fargo, North Dakota 58105.

<sup>1</sup>Present address: Midwest Research Institute, Chemical Sciences Division, Kansas City, MO 64110.

over the past three decades. This, in part, reflects the complexity of the herbicide's metabolism and the variation in activity of the enzymes which affect the compound in different plant species. As recently as 1977 a previously undiscovered metabolite of the herbicide was identified in plant tissue (Chkanikov et al., 1977), and there are yet plant metabolites of 2,4-D which have not been chemically