Effects of Processing on Levels of Morestan Residues Occurring in Papaya Purees

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Papayas (*Carica papaya* L.) were sprayed with the miticide Morestan (6-methyl-2,3-quinoxalinedithiol cyclic carbonate) at levels of 8 and 12 ounces of actual Morestan per 100 gallons of water. The sprayed fruits were processed into purees and the products were analyzed for Morestan residues by gas chromatography. Treatment of the fruit with hot water prior to simulated commercial processing removed about 11% of the residue present on the fruit. Purees processed from peeled fruit contained Morestan residues of 0.2 to 0.3 p.p.m. compared to residues of 1.0 to 2.4 p.p.m. present in purees made from the whole fruit.

A recently completed field study showed that Morestan (6-methyl-2,3-quinoxalinedithiol cyclic carbonate) was effective for the control of the red and black flat mite, *Brevipalpus phoenicis* (Geijskes) (Acarina: Tenuipalpidae), on the Solo variety of papaya (*Carica papaya* L.) (Haramoto, 1966). Sufficient analytical data were obtained to show that Morestan residues were present only on the surface of the papayas; no residues occurred in the peeled edible part of the fruits. Prior to the shipment of the fresh fruit or the processing of the fruit into puree, newly harvested papayas are usually immersed in water at 50° C. for 20 minutes to prevent or retard the development of postharvest decay (Akamine and Arisumi, 1953) that would otherwise occur during the subsequent 3- to 5-day storage period. The water treatment should also remove extraneous material from the fruit, including pesticide spray deposits. It is common practice in the commercial preparation of papaya purees to comminute the washed whole fruit; the peel becomes an integral part of the pulp and it may provide a source of pesticide contamination in the final product. Therefore, Morestan-treated papayas were obtained from a controlled experimental field plot area, processed into purees, and analyzed for Morestan residues to determine the amount of residue removed by washing and processing.

MATERIALS AND METHODS

Source of Papayas. Three plots of papaya trees, 18 trees in each plot, located at the Hawaii Agricultural Experiment Station Farm at Poamoho, Oahu, were used for this study. Morestan, a compound insoluble in water and marketed as a 25% wettable powder, was applied to the papayas in the form of a suspension in water. The fruits from one plot of trees supplied the control samples (no Morestan was applied in this area); a second plot supplied the samples from trees sprayed with a formulation which contained 8 ounces of actual Morestan per 100 gallons of water; a third plot supplied samples treated with 12 ounces of actual Morestan per 100 gallons of water. The pesticide formulations were applied with a power sprayer under 200 p.s.i. and each tree was covered with sufficient spray to the point of run-off. Three spray applications were made to the trees at intervals of approximately one month between applications. Weather conditions governed the dates of application, which were August 28, September 21, and October 24, 1967. Application to the U. S. Department of Agriculture for label registration for the use of Morestan on papayas is now in process; the maximum amount of the compound to be recommended for use on this commodity will most probably be no greater than 4 ounces of actual Morestan per 100 gallons of water per acre. Fruits were sampled from the control and treated trees after the third application of the pesticide. A maximum sampling of two fruits were obtained from each tree, with an average number of 25 fruits (15 to 25 pounds) from each plot on each sampling date. Two papayas were retained from each sample for laboratory studies, and the balance of the fruit was delivered to the USDA Hawaii Fruit Laboratory for puree processing. Morestan-treated fruits were also obtained from the Hawaii Agricultural Experiment Station Farm at Waimanalo, Oahu, for supplementary residue studies pertinent to the hot-water treatment of papayas prior to processing.

Laboratory Processing of Whole Papaya Fruit. Papayas were quartered and opposite quarters were finely chopped and composited, including the peel and seeds. These samples were not pretreated with hot water. Fifty-gram samples of the composites were weighed into quart-size

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Mason jars, 250 ml. of redistilled acetone was added to each sample, and the jars were sealed and stored until needed for extraction and residue analysis.

Additional fruits, obtained from Poamoho and also from the Experiment Station at Waimanalo, Oahu, with variable Morestan pesticide application and harvest histories, were soaked in containers of water at different temperatures and different time periods as listed in Table I. The fruits were removed from the water, drained, and prepared for extraction as described above. Each of the water samples (2000 to 2500 ml. per papaya) in which the fruits had been soaked was extracted three times with 25-ml. portions of hexane; the hexane extracts were combined, filtered through a plug of anhydrous sodium sulfate, concentrated on a steam bath to a suitable volume, and analyzed for Morestan residue by gas chromatography as described below.

Simulated Commercial Processing of Papaya Purees. Approximately 9 kg. (20 to 30 fruits) of papayas of each sampling was soaked for 20 minutes in 90 liters of water maintained at 50° C. in a covered stainless steel kettle. The fruits were removed from the water, drained, and stored for 3 to 5 days in paper-lined wooden crates at room temperature (25° to 30° C.) for ripening. The ripe fruit of each sample was divided into two equal lots. The whole fruit (including the peel) of one lot was macerated with a machine comparable to the Model D Comminuting machine designed by the Fitzpatrick Co., Chicago, Ill. The fruit of the other lot was sliced into one-third radial lengthwise segments; the segments were peeled, prior to maceration, with a pilot model machine developed by the Agricultural Engineering Department of the University of Hawaii (Stafford *et al.*, 1966). The macerated whole fruit and peeled fruit were processed through equipment containing an 0.83-mm. screen and then through a 0.50-mm. screen, commercially designated as a pulper and finisher, respectively (equipment produced by the Langsenkamp Co., Indianapolis, Ind.). This procedure removed and discarded the seeds and coarse fibrous particles of the fruit and produced a uniform, fine-particled pulp. The pulps were packed in No. 2 enameled cans, sealed, and heated in a boiling water bath for 12 minutes. The canned purees were chilled in iced water immediately after removal from the hot water bath and stored in the freezer until needed for residue analysis.

One-liter samples of the soak water in the stainless steel kettles were retained and prepared for Morestan analysis as described above for the laboratory-process water.

Analytical Procedure for Morestan Residue Analysis. The analytical procedure, with minor modifications, was that recommended by Anderson (1967) for the analysis of Morestan residues in crops. Before use, the organic solvents used in this study were redistilled in an all-glass system, and the sodium sulfate was washed with petroleum ether and dried overnight at 130° C. Florisil, 60- to 100-mesh, was heated 16 hours at 130° C. prior to use. Analytical grade Morestan was obtained from the Chemagro Corp., Kansas City, Mo.

Apparatus. Chromatographic tubes, borosilicate glass, 20 × 400 mm., with Ultramax stopcocks and 300-ml. reservoirs.

Gas chromatograph, Aerograph Model 204-B, electron-capture detector (250 mc.); 5-feet × 1/8-inch glass spiral column packed with 3% SE-30 silicone on Chromosorb W (AW, DMCS), 80- to 100-mesh; column temperature 185° C., injection temperature 200° C., detector temperature 190° C.; nitrogen carrier gas, flow rate 30 ml. per minute; Leeds and Northrup Model H, 1-mv. full scale recorder, chart speed 0.5 inch per minute.

Preparation of Standard Morestan Curve. A stock solution was prepared to contain 1 mg. per ml. of Morestan in petroleum ether. Solutions containing 0.1, 0.2, 0.3, 0.4, and 0.5 ng. of Morestan per 5 µl. of petroleum ether were prepared from the stock solution and were used to obtain the gas chromatograph calibration curve for this compound. The standard curve was linear between

Table I. Morestan Residue Removed from Papayas by Water Wash, Under Laboratory Processing Conditions, Using Variable Conditions of Time and Temperature

	Water Wash Conditions			Residue, 8-Ounce Level						Residue, 12-Ounce Level				
Fruit History	Min.	°C.	Fruit Wt., Grams	On fruit, µg.	In water, µg.	Total µg.	% removed by water	Av. % removed by water	Fruit Wt., Grams	On fruit, µg.	In water, µg.	Total, µg.	% removed by water	Av. % removed by water
Harvested 0 days	10	25	551	116	33	149	22.1		401	192	60	252	23.8	
after first appli-	10	25	407	419	31	450	6.9	14.5	566	742	75	817	9.2	16.5
cation of More-														
stan	10	50	371	78	32	110	30.0		370	222	92	314	29.3	
	10	50	511	204	33	237	13.9	22.0	354	258	45	303	14.8	22.0
Harvested 30 days	20	50	477	57	7	64	10.9		...	...	...	...	...	
after first appli-	20	50	481	91	5	96	5.2		463	102	18	120	15.0	
cation of More-	20	50	495	59	3	62	4.8		469	66	7	73	9.6	
stan	20	50	429	64	5	69	7.2		396	67	3	70	4.3	
	20	50	1573	94	49	143	34.3	12.5	1687	169	28	197	14.2	10.8
Harvested 0 days	20	50	615	572	42	614	6.8		818	1309	90	1399	6.4	
after second ap-	20	50	622	435	50	485	10.3		1022	1022	152	1174	12.9	
plication of	20	50	636	114	22	136	16.2		850	672	140	812	17.2	
Morestan	20	50	582	326	65	391	16.6	12.5	766	505	145	650	22.3	12.2

0.1 and 1.0 ng. of Morestan for a given attenuation of the electrometer, ordinarily set at a range of 10 and an attenuation value of 2.

Preparation of Samples for Morestan Residue Analysis. Fifty grams of composited whole fruit, peeled fruit, peel, or puree was blended with 250 ml. of acetone in an Omni-Mixer for 3 minutes. The slurry was filtered through a Whatman No. 12 fluted filter paper. One hundred fifty milliliters of the filtrate was transferred into a 500-ml. separatory funnel which contained 150 ml. of petroleum ether. The mixture was shaken vigorously for 30 seconds, and allowed to stand until the aqueous and organic solvent phases had separated. The lower aqueous phase was discarded, the petroleum ether fraction was transferred to a 400-ml. beaker, and the funnel was rinsed with 50 ml. of petroleum ether into the same beaker. The extract was evaporated nearly to dryness on a steam bath with the aid of a jet of filtered air. The concentrated extract was quantitatively transferred with 30 ml. of benzene to a chromatography tube which contained 20 grams of Florisil topped with 10 grams of anhydrous sodium sulfate (the Florisil column was saturated, just prior to use, with benzene). The solution was percolated through the column at the rate of two drops per second. When the level of the solution reached the top edge of the sodium sulfate layer, 125 ml. of acetone was added to the column, elution was continued, and the acetone eluate was collected in a clean 250-ml. beaker; the benzene eluate was discarded. The acetone eluate was evaporated nearly to dryness on a steam bath, the concentrate was quantitatively transferred to a 25-ml. volumetric flask with petroleum ether and made to volume with the same solvent. Aliquots of 5 μl. of this sample solution were applied to the gas chromatograph for Morestan analysis.

RESULTS AND DISCUSSION

The nonuniform appearance of the Morestan spray deposits on the papayas suggested that any removal of the pesticide residue by the water-wash treatment would be of an empirical nature. The physical effects of handling and transporting the fruits would also govern the amount of residue on the fruits, especially if they were harvested prior to any extended period of exposure to the weather. Yellow solids were observed in the water samples obtained from the soaking kettles after immersion of the papayas (pure Morestan is a yellow crystalline powder); these yellow particles were partitioned into the organic solvent phase when the water was extracted with petroleum ether.

The empirical nature of the effect of soaking papayas in water on the removal of Morestan from the fruit is illustrated in Tables I and II. The duration of soaking and the temperatures of the bath waters could not be correlated with the amount of residue removed during the soaking period. The amount of residue removed by water treatment of the fruit, in the laboratory experiments, varied from 4 to 34%, with an average amount of 15% in both the 8- and 12-ounce pesticide levels. Under simulated commercial processing conditions, the water removed 8 to 16% of the residue, with an average amount of 11% in both the 8- and 12-ounce pesticide levels.

Data on Morestan residues found on the papayas prior to processing are given in Table III. No noticeable decline was observed in the residue levels on the fruits harvested between the period of 0 and 7 days after the final spray application. This may have been partly due to the low rainfall in the Poamoho area during the period of this study. Total rainfall was 0.50 inch for the 24-day period following the first application of the pesticide to the trees, 1.44 inches for the 33-day period following the second application, and 2.29 inches for the 28-day period after the third application. The temperature of the orchard area for this period of time ranged from a minimum of 21° C. to a maximum of 31° C. Data are also given in Table III on the Morestan residues found in the simulated commercially processed purees utilizing the whole fruit, including the peel layer. Whole fruit slurries prepared from nonwashed fruit by the laboratory process had average residue levels of 3.1 and 4.4 p.p.m. (8- and 12-ounce Morestan spray levels, respectively). Whole fruit purees prepared by the simulated commercial process (pretreatment of the fruit with water and removal of some peel due to pulping and finishing) had average residues of 1.1 and 1.7 p.p.m., a reduction of 64 and 61%, respectively, for fruit sprayed with the 8- and 12-ounce levels. Peeled fruit purees had average residues of 0.17 and 0.29 p.p.m., a reduction of about 93% (for both Morestan application rates) of the residue found on the raw fruit. Residue data on the 5- and 7-day samples of the peeled fruit purees were not attainable, because of incomplete ripening of the fruit caused by unusually low local temperature conditions during the period of fruit storage; it was impossible to efficiently peel the semiripe fruit. However, the overall residue data on the whole fruits indicated that the residue values for these samples would have been quite similar to the values obtained for the 0-, 1-, and 3-day samples. Recovery data, obtained by adding known amounts of Morestan to the samples, are shown in Table IV; recovery of the added pesticide was consistently good.

Earlier in this report, it was noted that no Morestan residue occurred in the peeled, edible part of the papaya. This observation referred to previous studies where extreme care was exercised in the removal of the peel to avoid pesticide contamination of the peeled fruit. Understandably, such care cannot be practiced in the commercial peeling process, and some contamination will be inevitable if pesticide residues are present on the outer surface of the

Table II. Morestan Residue Removed from Papayas by Water Wash Using Simulated Commercial Processing Conditions

Sampling Time after Final Application of Morestan, Days	8-Ounce Level			12-Ounce Level		
	Residue in whole fruit before water wash, p.p.m.	Residue Removed by Water		Residue in whole fruit before water wash, p.p.m.	Residue Removed by Water	
		P.P.M.	%		P.P.M.	%
0	2.0	0.3	15	5.2	0.5	10
1	3.4	0.3	9	4.0	0.4	10
3	3.6	0.3	8	4.8	0.4	9
5	2.4	0.4	16	4.6	0.7	15
7	4.2	0.4	9	3.2	0.3	9
Average			11			11

Table III. Morestan Residues in Papaya Puree Processing

Sample Material	Morestan Applied, Oz./100 Gal. Water	Sampling Time (Days) after Final Application of Morestan					
		0	1	3	5	7	Average
		Residue, P.P.M.					
A Whole fruit, not prewashed with water	8	2.0	3.4	3.6	2.4	4.2	3.1
	12	5.2	4.0	4.8	4.6	3.2	4.4
B Whole fruit puree, simulated commercial process	8	1.3	1.0	1.2	0.8	1.4	1.1
	12	1.6	1.6	1.7	1.2	2.4	1.7
C Peeled fruit puree, simulated commercial process	8	0.16	0.16	0.20	...	...	0.17
	12	0.23	0.34	0.30	...	...	0.29
Apparent residue removed during processing of whole fruit (A-B)	8						2.0
	12						2.7
Apparent residue removed during processing of peeled fruit (A-C)	8						2.9
	12						4.1

Table IV. Recovery of Morestan

Sample	Morestan Added, P.P.M.	Average Recovery, %
Wash water 25° C.	0.1	86
	0.5	86
	1.0	90
50° C.	0.5	90
	1.0	85
Whole papaya fruit	0.1	94
	0.5	94
	1.0	93
Papaya puree	0.1	100
	0.1	88
	0.1	96
	0.1	92

fruit. For example, the peeling machine used in this study consisted essentially of two reels, which rotated at different speeds, with the papaya slices fed between them. The upper wooden reel was covered with a thin sheet of corrugated rubber; the larger lower reel, which separated the peel from the flesh, was made of stainless steel rods 0.3 inch apart. Obviously, the fruit slice will be subjected to pressure and friction when it is peeled and some of the Morestan spray deposit will be removed; the residue will adhere to the rollers of the machine, it will eventually be mixed with the peeled fraction of the fruit, and a portion of this residue will terminate in the final puree product, as indicated by the data presented herein. However, peeling the fruit prior to processing effected approximately a sevenfold decrease in the amount of Morestan residue present in the final puree product.

Rainfall was abnormally low in the field plot areas used for this study. Ordinarily, considerably greater amounts of rain could be expected in papaya-growing areas prior to harvest, which would contribute to the removal of some of the residue from the fruit. Also, the application rates of 8 and 12 ounces of Morestan per 100 gallons of water are greater than the amounts that will be recommended for use, which will probably be a maximum of 4 ounces of Morestan per 100 gallons of water; therefore, any residues on the fruit would be decreased proportionately.

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