i.e. 4-hydroxycarbaryl glucuronide—conjugated through the naphthyl moiety fluoresces at pH 7.5 (335 mμ) and is nonfluorescent at pH 11 (465 mμ), while metabolite *D* (carbaryl *O*-glucuronide) fluoresces at pH 11 (465 mμ), but is nonfluorescent at pH 7.5. Conjugation of carbaryl through the *N*-hydroxy group produces a molecule that has fluorescent properties similar to carbaryl, while metabolite *D* has fluorescent properties similar to *N*-acetylcarbaryl. Metabolite *C* displays fluorescent properties similar to *D* and *N*-acetylcarbaryl. At the present time the identity of metabolite *C* is unknown.

Thirty-nine to 44% of the administered carbaryl was hydrolyzed and the liberated 1-naphthol conjugated with glucuronic and sulfuric acids. Fluorometric and chromatographic analysis of urines obtained from men exposed to carbaryl dust during packaging operations yielded additional evidence for conjugation after hydrolysis.

Fluorescence was found to be useful during radioactive studies in confirming the presence of the naphthyl moiety by direct (4-hydroxycarbaryl glucuronide, naphthyl glucuronide, 4-hydroxycarbaryl sulfate, naphthyl sulfate) or indirect (carbaryl *O*-glucuronide) analysis. Fluorescence analysis combined with established chromatographic procedure made possible the identification of the two principal metabolites of carbaryl in man.

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RESIDUES IN CITRUS

2,2-Dichloropropionic Acid Residues in Citrus Fruit from Florida Following Applications of the Herbicide

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Dalapon residues in citrus fruits from Florida following a variety of Dowpon applications ranged from 0 to 11 p.p.m. The recovery of added dalapon was 90% over the range of 0.2 to 20 p.p.m. The residual herbicide in a sample appeared to be related to several factors including the actual amount of chemical applied. A maximum residue of 5 p.p.m. may result from treatments that are recommended on the label. A study related to time between application and harvest indicates that the greatest residue in fruit is found at the time of a flush of growth of the trees. No loss of chemical was caused by a heat treatment simulating commercial pulp drying. Residues from ditch-bank treatments were 0.2 p.p.m. and lower.

THE PREVALENCE of many species of grass in areas where citrus fruit is grown causes a very difficult control problem. Bermuda (*cynodon dactylon*), bahia (*Paspalum notatum*), para (*Panicum purpurascens*), guinea (*Panicum maximum*), maidencane (*Panicum hemitomon*), and Johnson (*Sorghum halepense*) grasses are among the principal offenders, competing with the citrus trees for water and nutrients. Uncontrolled grass growth makes other pest control difficult, and adds greatly to grove maintenance problems. By the proper use of Dowpon (The Dow Chemical Co.) containing 85% of the sodium salt of dalapon (trade-mark of The Dow Chemical Co. abroad for 2,2-dichloropropionic acid), it is possible to control most grasses safely with a minimum of labor.

This study was undertaken to determine the residue of dalapon in citrus fruit from areas treated with Dowpon for control of grasses.

The data presented here were used in a petition to the U. S. Food and Drug Administration to establish a tolerance for dalapon in citrus fruits from Florida (*7*). As a result of this work, Dowpon is accepted for use in commercial orchards in Florida with a legal tolerance of 5 p.p.m. of dalapon in grapfruit, limes, tangerines, and oranges.

Experimental

Applications were made as indicated in Tables II, III, and IV. In general, each experiment consisted of treatment of plots of 2 to 6 trees replicated at least twice. A sample was taken by random selection of 4 to 10 oranges from each tree, which were combined into a single sample. When duplicate samples were collected, the process was repeated.

The samples were shipped to the

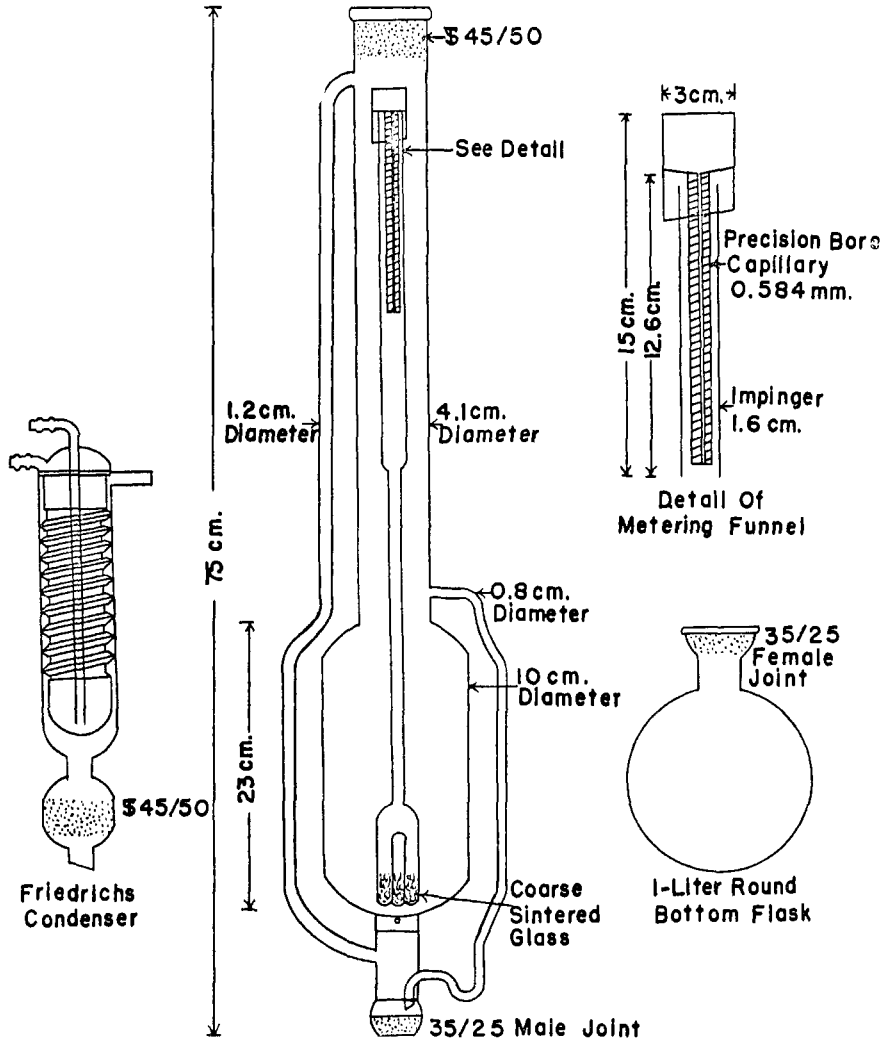

Figure 1. Continuous liquid-liquid extractor

laboratory in Midland, Mich., and held under refrigeration at 37° F. until they were ground in a meat grinder within a few days. The pulp was well mixed and aliquots were put into polyethylene bags and stored in a deep freeze at 0° F. until they were analyzed.

Analytical Procedure

Residues of dalapon were determined in two laboratories by three analysts. The basic procedure used is that described by Smith, Getzendaner and Kutschinski (*2*). Because of the nature of the samples, the following extraction step was introduced.

A 100-gram sample of finely ground fruit was extracted with 150 ml. of water in a Waring Blendor. Fifteen milliliters of concentrated HCl or 85% H_3PO_4, 25 ml. of 25% phosphotungstic acid solution, and about 40 grams of Hyflo Supercel were added with constant stirring. The slurry was filtered on a pad of Supercel and washed well with water until a volume of 600 ml. was reached. This was extracted as indicated.

The ether extract, when transferred to a separatory funnel, was accompanied by a sirupy heavier phase, which was discarded. The ether phase was washed once with a small quantity of water, which was also discarded. The ether phase was then extracted with caustic, and the procedure for determination of residues in sugar cane was followed (*2*).

For some extractions, a continuous ether extractor was used which was different from the extractor previously described. Figure 1 gives the details of its construction.

For determination of residues in dried pulp, a 25-gram sample was soaked in 300 ml. of water for an hour to soften it before blending.

Results and Discussion

The data obtained for verification of the method by the two laboratories are given in Table I. The three analysts recovered an average of 90 ± 14% (95% confidence limits) of dalapon added to four species of citrus fruits, orange juice, and dried orange pulp over the range of 0.2 to 20 p.p.m.

Data on citrus fruit samples analyzed in duplicate are summarized in Tables II and III. Untreated control samples ranging from 0.0 to 0.3 p.p.m. of apparent dalapon, averaged 0.1 p.p.m.

Table II summarizes residue values found in grapefruit, limes, oranges, and tangerines from a variety of application and harvest schedules. As experience was gained through the field experiments, rates of application reflected the development of practical schedules with regard to economically feasible amounts and timing for control of grass. It was initially assumed that massive dosages would be required, such as 10 and 20 pounds per acre applied three times at 2-week intervals. However, experience shows that three applications of 2 to 4 pounds per acre each at 2- to 10-day intervals give adequate grass control and are more economical. Residues found at at the highest rates of use did not exceed 11 p.p.m. No residue was found

Table I. Recovery of Known Amounts of Dalapon Added to Citrus Fruit Samples

Sample	Dalapon, P.P.M. Added	Dalapon, P.P.M. Found	Recovery, %
Grapefruit	0.20	0.16	80
		0.18	90
	0.20	0.16	80
		0.20	100
Oranges	0.20	0.17	85
		0.18	90
Tangerines	0.20	0.17	85
		0.16	80
Grapefruit	0.50	0.50	100
		0.51	102
		0.44	88
		0.44	88
Limes	0.50	0.53	106
		0.42	84
Oranges	0.50	0.46	92
		0.49	98
		0.40	80
Grapefruit	1.0	1.01	101
		0.99	99
		0.87	87
		0.80	80
Limes	1.0	1.01	101
		0.84	84
Oranges	1.0	0.87	87
		0.89	89
Grapefruit	2.0	1.76	88
		1.70	85
Limes	2.0	1.80	90
Oranges	2.0	1.77	89
		1.83	91
Tangerines	2.0	1.82	91
		1.64	82
Limes	2.5	2.60	104
Orange juice	3.0	2.60	87
Grapefruit	5.0	4.99	100
		4.94	99
		4.57	91
		4.55	91
Limes	5.0	5.09	102
Oranges	5.0	4.43	89
		4.45	89
Orange skins pulp	5.0	4.4	88
Orange juice	5.0	4.3	86
Tangerines	5.0	4.27	85
		4.46	89
Grapefruit	7.5	7.28	97
Limes	7.5	7.60	101
Grapefruit	10.0	10.0	100
Orange skins pulp	10.0	8.7	87
Dried pulp	15.0	12.2	81
	20.0	16.7	83
		17.2	86
		17.2	86
		18.0	90

Average recovery, 90%
Standard deviation, 7%

Table II. Residue of Dalapon Found in Florida Citrus Fruit from Plots Treated with Dowpon

Variety	Location	Dates		Total Dowpon Applied, Lb./Acre	Samples[a] Analyzed	Dalapon Found, P.P.M.[b]
		Application	Harvest			
		GRAPEFRUIT				
Excelsior	Lake Alfred	11/23, 25, 27/59	12/11/59, 1/22/60	0	4	0.1
				6[c]	8	0.1–0.2
Marsh, seedless	Lake Alfred	6/21, 29/56	11/21/56	0	1	0
				20[d]	1	0.2
Marsh, seedless	Ft. Pierce	7/17, 30; 8/13/58	10/2/58	0	2	0.1–0.2
				30[e]	1	0.4
				60[e]	1	0.3
Pink seedless	Merritt Is.	4/13, 15, 17/59	12/7/59	0	1	0
				6[c]	1	0
Ruby Red	Lake Wales	4/5, 19; 5/3/57	10/16/57	0	2	0.0–0.1
				30[d]	2	0
				60[d]	2	0
Ruby Red	Clemont	4/2; 5/3/56	10/20/56	0	1	0.1
				20[d]	1	8.0
		LIMES				
Persian	Crooked Lake	4/18/56	11/21/56[f]	0	1	0
				5–12[c]	4	0–0.2
		ORANGES				
Hamlin	Waverly	4/5, 19; 5/3/57	10/16/57	0	2	0.1
				30[d]	2	0.4–0.6
				60[d]	2	1.6–1.8
Hamlin	Lake Alfred	4/28, 30; 5/2/59	11/30/59	0	1	0.1
				6[g]	3	0–0.1
Hamlin	Lake Alfred	11/23, 25, 27/59	12/11/59 to 3/18/60[h]	0	7	0.1
				6[g]	18	0.1–0.8
Parson Brown	Lady Lake	3/6, 21, 25/59	12/11/59	0	2	0.2
				4–6[g]	2	0
Pineapple	Lake Wales	7/16, 28; 8/12/58	10/3; 12/30/58[i]	0	5	0.1–0.3
			12/11/59 to	30[g]	8	1–11
Pineapple	Lake Alfred	11/23, 25, 27/59	3/18/60[h]	0	7	0.1–0.2
				6[g]	18	0–1.5
Pineapple	Orlando	4/15, 17, 19/59	11/7/59	0	1	0.1
				6[g]	2	0
Pineapple	Mimms	4/13, 15, 17/59	12/7/59	0	1	0.1
				6[g]	1	0
Temple	Winter Haven	5/18, 21, 23/59	12/30/59	0	1	0
				6[g]	3	0–0.2
Valencia	Ft. Pierce	3/2, 4, 6/59	12/28/59	0	1	0.1
				6[g]	2	0
Valencia	Lake Alfred	1/18, 20, 22/60	2/8; 6/8/60[h]	0	8	0.1
				6[g]	30	0–0.6
Valencia	Avon Park	3/28; 4/11/57	1/9/58	0	3	0.1
				10[j]	3	0
				20[j]	3	0.2–0.3
		7/17, 27; 8/12/58	10/3/58	0	3	0.1
				30[g]	2	1.5–2.2
		TANGERINES				
	Waverly	4/5, 19; 5/3/57	10/16/57	0	2	0.1
				30[d]	1	0
				60[d]	1	0.2
Dancy	Lady Lake	3/6, 21, 25/59	12/11/59	0	1	0.1
				6[g]	1	0

[a] All samples analyzed in duplicate. [b] Blank value subtracted from treated samples. Values corrected for 90% recovery. [c] Directed spray to include entire root zone. [d] Sprayed to wet Bermuda grass in 8- × 8-foot plot around base of each tree. [e] Broadcast spray to wet entire ground around area between trees. [f] Two months before normal harvest. [g] Broadcast spray to wet grass. [h] For plot of residue *vs.* harvest date (Figure 2). [i] For details see Table IV. [j] Power hand gun covering 25- × 75-foot plot except for 4- × 4-foot area around base of each tree.

in some of the samples from plots receiving a total of 60 pounds per acre in three applications.

Table III gives data on samples from trees in groves where ditch banks were treated with Dowpon. Residue content is very low in all cases.

The highest residue reported in Table II was 11 p.p.m. in pineapple oranges. Details of this experiment, given in Table IV, show that, during the normal harvest period, the residues were lower than one and two months before normal harvest, from an application schedule of 10 pounds per acre applied three times. The last application was about $2^1/_2$ months before the first sampling. Dalapon residues found in oranges from groves treated with a total of 15 pounds per acre were considerably lower than residues in oranges from groves treated with 30 pounds per acre.

Fruit from the October 27 orange harvest were juiced and herbicide residues were determined on the rag and peel as well as in the juice. The juice contained only about half as much dalapon as the rag and peel fraction (Table IV). This difference is probably caused by the acid condition of the orange, in which the dichloropropionic acid would exist in the free acid form, which is more soluble in the oils of the skin than in the aqueous portions.

Because Table IV indicated the possibility of a build-up and decline of residue over a period of time, this effect was studied in greater detail. Applications of Dowpon were made to oranges of the Pineapple, Hamlin, and Valencia varieties and to Excelsior grapefruit at the rate of 2 pounds per acre repeated three times at 2- to 4-day intervals. The herbicide was applied as sprays directed to the under-tree area to include

Table III. Residue of Dalapon Found in Florida Citrus Fruit from Plots Where Ditches Were Treated with Dowpon

Variety	Location	Dates: Application	Dates: Harvest	Total Dowpon Applied, Lb./Acre	Samples[a] Analyzed	Dalapon Found, P.P.M.[b]
		GRAPEFRUIT				
Gentile	Ft. Pierce	6/15/59	1/27/60	0	1	0.1
				8[c]	3	0–0.2
Marsh	Van Beach	6/4, 15; 8/14/59	1/27/60	0	1	0.1
				25[d]	3	0
		ORANGES				
Pineapple	Van Beach	6/4, 15; 8/14/59	1/27/60	0	1	0.1
				25[e]	3	0
Valencia	Ft. Pierce	9/1/59	1/22/60	0	1	0.04
				5[f]	3	0.1
Valencia	Vero Beach	6/4, 15; 8/14/59	1/23/60	0	1	0.1
				25[e]	3	0

[a] All samples analyzed in duplicate. [b] Blank values subtracted from treated samples. Values corrected for 90% recovery. [c] Directed to weeds in ditches and ditch bank in 300 to 600 gal./acre. Trees 6 to 9 feet. [d] Directed to weeds in ditches. Trees 12 feet from ditches which are on three sides of each tree. Dowpon applied at rates of 10, 10, and 5 lb./acre. [e] Directed to weeds in ditches and ditch bank. Tree rows 3 to 4 feet from treated ditch. Dowpon applied at rates of 10, 10, and 5 lb./acre. [f] Directed to weeds in ditches and ditch bank. Tree rows 17 feet from treated ditch.

Table IV. Residues of Dalapon in Florida Oranges

Source: Lake Wales
Application: 7/16, 7/28, 8/12/58. Broadcast on 12- × 12-foot area around base of each tree. Sprayed to wet grass. Three applications.
Samples: 10 fruits from each of 3 trees per sample.
Variety: Pineapple, 5-year old trees
Normal harvest: December to February[a]

Sample	Application of Dowpon: Lb./acre/applic.	Application of Dowpon: No.	Sampling Date	Apparent Residue of Dalapon, P.P.M.: Gross	Net	Corrected[b]
			ORANGES[a]			
4-A	0	...	10/3/58	0.1		
				0.1		
4-B	0	...	10/3/58	0.1		
				0.1		
				0.3		
4-C	0	...	10/27/58	0.1		
				0.1		
LW 4-A	0	...	12/30/58	0.1		
				0.1		
LW 4-C	0	...	12/30/58	0.3		
				0.3		
			Av.	0.2		
3-A[a]	10	3	10/3/58	9.9	9.8	11
				9.4	9.3	11
3-B[a]	10	3	10/3/58	9.5	9.4	11
				9.2	9.1	10
3-C[a]	10	3	10/27/58	8.6	8.5	9
				9.2	9.1	10
				8.8	8.7	10
LW 3-A	10	3	12/30/58	6.6	6.4	7
				6.4	6.2	7
LW 3-B	10	3	12/30/58	4.1	3.9	4
				4.2	4.0	5
LW 3-C	10	3	12/30/58	1.9	1.7	2
				1.4	1.2	1
				1.6	1.5	2
LW 1-A	5	3	12/30/58	1.2	1.0	1
				1.2	1.0	1
LW 1-B	5	3	12/30/58	1.2	1.0	1
				1.1	0.9	1
			ORANGE JUICE[c]			
4-C	0	...	10/27/58	0.1		
				0.1		
3-C	10	3	10/27/58	4.1	4.0	5
				4.1	4.0	5
			ORANGE PEEL AND RAG[c]			
4-C	0	...	10/27/58	0.1		
				0.1		
3-C	10	3	10/27/58	6.9	6.8	8
				9.1	9.0	10
				8.6	8.5	9

[a] Oranges sampled 10/3 and 10/27/58 were taken before normal harvest. [b] Corrected for recovery of 90%. [c] Juice and peel and rag samples numbered 4-C and 3-C are from oranges having the same sample designation in this table.

the entire root zone. Samples were taken from these plots intermittently over an extended period during the normal harvest time. The data on residues as summarized in Table II are plotted in Figure 2, which shows the relationship between data of harvest and maximum residue level for oranges.

In Figure 2 the data are plotted to show the residue at time intervals following application. The greatest residue concentration occurred while the trees were in a flush of growth, rather than being related directly to the period between last application and harvest. Harvest in this experiment was from 55 to 100 days after application, depending on the variety. Grapefruit samples were not collected for as long a period as the oranges because of the scarcity of fruit in the plots.

Since it is the practice in Florida to dry citrus pulp for cattle feed, an experiment was carried out to determine the effect on dalapon content of drying pulp from oranges containing 8 to 10 p.p.m. of residue. A dryer was constructed which approximated conditions used to dry citrus pulp commercially. This consisted of a sheet metal cylinder 6 inches in diameter and 14 inches long, mounted horizontally on a rotating yoke driven at 10 r.p.m. Screens were fitted into the bottom and top, and a cylinder of screen about 4 inches in diameter was mounted inside the sheet metal cylinder concentric with it. Hot air was blown through this

Table V. Moisture Content of Fresh and Dried Orange Pulp

Sample	Moisture, %: Fresh	Moisture, %: Dried
11451	77.0	5.11
	76.9	6.32
11452	76.4	5.25
	...	6.24

Table VI. Effect on Dalapon Residue of Drying Orange Pulp

Sample	Apparent Residue of Dalapon, P.P.M. Fresh	Dried Grass	Dried Net	Dried Corrected[a]	Residue Expected in Dry Pulp[b] (Calcd.)
		UNTREATED			
11452	0	1.8			
	0	1.4			
	Av.	2.0			
		TREATED			
11451	7	20	18	21	18
	9	15	13	15	18
	9				14
				Av. 18	17

[a] Corrected for 85% recovery.
[b] Calculated from residue found in fresh pulp, amount of molasses added, and loss in weight.

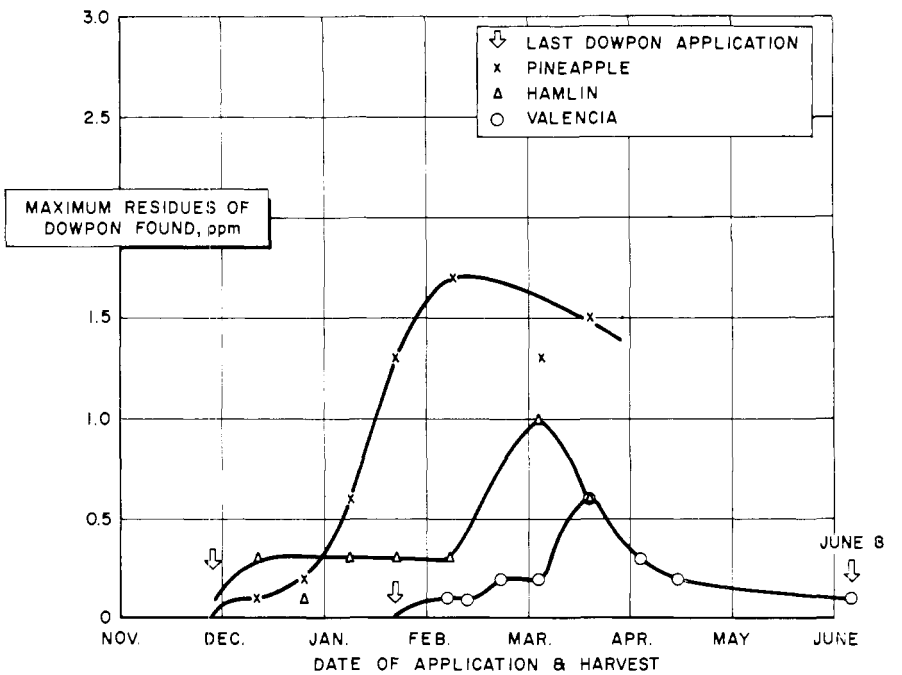

Figure 2. Residue of dalapon in oranges at various dates following Dowpon applications

from a variable 1000-watt heating element by a variable-speed fan mounted behind the heater. Air stream temperatures up to 235° C. are attainable with this dryer. The whole cylinder was wrapped with asbestos paper for insulation. A metal thermometer was inserted through the wall to measure the temperature inside the chamber.

Samples were introduced into the screen cylinder for drying. Heated air was passed through the rotating cylinders at maximum temperature until an internal temperature of about 135° C. was reached (about 8 minutes). The pulp was then dried for 20 minutes as the temperature was gradually reduced to 70° to 80° C.

The procedure for preparing the pulp was copied after commercial practice. Combined skins, pulp, and seeds, obtained by removing juice as described above, were mixed with lime at the rate of 3.2 grams per kilogram. Juice amounting to 10% of the mass was removed from this. Sugar cane molasses of about 75% sugar content was added to bring the juice sugar content up to 65%, and the juice was mixed back with the solids. The mixture was then dried as described above.

The molasses used in commercial practice results from the partial dehydration of some of the juice removed after the addition of lime. This is added to the remaining juice at the proper rate to give the desired sugar content. Since orange molasses was not available, sugar cane molasses was substituted.

The amount of moisture in the fresh pulp and the dried product was determined by drying at 70° C. under 10-cm. pressure for 5 and 4 hours, respectively.

The moisture was reduced from about 77% in the fresh material to about 6% in the dried product (Table V).

Recoveries of known amounts of dalapon added to the dried product from previously untreated plots are shown in Table I. In these experiments the dalapon was added after the pulp was dried, at 15 and 20 p.p.m. The average recovery is 85%.

The experimental drying probably does not reproduce conditions of regular commercial dryers where an oil-fired flame is introduced directly into the rotary chamber and the heat dries the pulp in about 20 minutes from the time it enters the dryer. Thus, pulp is heated to a higher temperature in actual practice than was used experimentally, and this might result in some loss of dalapon, although Table VI shows that in this experiment there was no loss.

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