An Analysis of Crop Sugars in the Oriental Fruit Fly, *Dacus dorsalis* Hendel (Diptera: Tephritidae), in Hawaii, and Correlation with Possible Food Sources[1]

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The main sources of food for oriental fruit fly adults (*Dacus dorsalis* Hendel) appear to be the juices and sap from intact or decaying plants, insect honeydew, and plant nectars (Nishida, 1958; Bateman, 1972). These natural products are high in carbohydrate content, invariably containing the common plant sugars sucrose and fructose, as well as glucose and a number of other sugars and sugar alcohols (Whiting, 1970). The high sugar content most likely serves the adult fruit fly as a source of energy for flight as is common in many of the Diptera (see the review by Sacktor, 1965).

In the Diptera, the crop serves as a storage organ, the contents usually undergoing little or no change (House, 1974). There also appears to be a lack of food absorption from this organ, the main site of uptake being the mesenteron, especially the gastric caecae (Treherne, 1957).

A qualitative chemical analysis by gas chromatography of the sugars present in the crops of adult oriental fruit fly males captured at different locations on Oahu, Hawaii, was made and the data used in an attempt to correlate the sugar profile of the crop with those from juices and sap collected from selected fruits found at the site of fly capture.

MATERIALS AND METHODS

Insects

Oriental fruit fly males were attracted in the field by the use of methyl eugenol (3, 4-dimethoxyallylbenzene) at three sites on Oahu in April, 1976: (1) A guava orchard at the University of Hawaii Agricultural Experiment Station Farm at Waimanalo, (2) Mt. Tantalus, in the vicinity of the Round Top, and (3) Lyon Arboretum, in upper Manoa Valley. Fruit flies were also obtained from the USDA Fruit Fly Investigations Laboratory in Manoa Valley for comparative analysis. The attracted fruit flies were subsequently collected with an insect net and immediately immobilized on ice to prevent possible sugar degradation in the crops.

Preparation of crop contents for sugar analysis

Fruit flies from a particular location were placed collectively in a Petri dish sitting on ice. With the use of a pair of fine tweezers, each fly was

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grasped at the posterior position of the metasternum and tip of the abdomen and the body gently pulled apart. Under the dissecting microscope, an engorged crop could easily be discerned as a small, globose bead along the length of the foregut (Fig. 1). The crop was then pierced with the drawn-out tip of a Pasteur disposable pipet and the contents aspirated and transferred to a 1 ml glass microvial on ice. The individual crop contents from each of the four locations were pooled. Approximately 20 full crops were sufficient to obtain a volume of 10-15 μ l. Approximately 50 insects were used per sampling site.

Derivatization of the crop contents for gas chromatographic analysis

In order to remove protein from the crop contents, enough trichloroacetic acid (0.6N) was added to the vial contents (15-20 μ l), to bring the total volume to 1 ml. The vial contents were then vigorously shaken, then centrifuged at 2000 RPM for 15 minutes. The supernatant was transferred to another vial and the solutes concentrated under a stream of nitrogen.

One milliliter of N-trimethylsilylimidazole (1.5 meq/ml) in hexane was added to each of the four samples, and the vials placed in a water bath at 60°C for 10 minutes. The supernatant was transferred to another vial and the solutes concentrated under a stream of nitrogen.

One milliliter of N-trimethylsilylimidazole (1.5 meq/ml) in hexane was added to each of the four samples, and the vials placed in a water bath at 60°C for 10 minutes. One milliliter of distilled water was added to the reaction mixture, the vial shaken, and the contents allowed to partition between organic and aqueous phases. The samples were again centrifuged at 2000 RPM for 15 minutes and the upper phase containing the TMS-sugar derivatives transferred to a new vial for gas chromatographic analysis. The analysis of sugars was based on a modification of the method developed by Brobst and Lott (1966) in which the sugars are converted to volatile trimethylsilyl (TMS) ether derivatives by the use of a reagent (N-trimethylsilylimidazole) which is specific for hydroxy and polyhydroxy groups, as occur in carbohydrates.

Juices analyzed were obtained from guava (*Psidium guajava* L.), papaya (*Carica papaya* L.), mango (*Mangifera indica* L.), pomegranate (*Punica granatum* L.), and surinam cherry (*Eugenia uniflora* L.) collected in the vicinity of fruit fly capture. Preparation of TMS-sugar derivatives from these juices were identical to that described above.

Gas chromatographic analysis

The derivatized sugar samples were analyzed in a Hewlett-Packard 5700A gas chromatograph equipped with a flame ionization detector and a 10 ft. x 1/8″ glass column containing 3% OV-17 on Chromosorb W(HP), 80/100 mesh. Operating parameters were: Carrier gas (nitrogen) flow rate, 40 ml/min with an inlet pressure of 60 PSIG; hydrogen and air inlet pressures were 15 and 24 PSIG respectively; detector temperature, 250°C. Analyses were programmed at the rate of 2°C/min with an initial temperature of 100°C and a final temperature of 220°C with a 16 minute hold. The strip chart recorder (Hewlett-Packard 7129A) was operated at the rate of 0.25 inches/min.

The following TMS-sugar standards in hexane (10%, w/v) were obtained commercially (Sigma Chemical Co., St. Louis, Mo.). Methyl eugenol and glycerol were derivatized in the laboratory.

L(+) arabinose
α-L(+) rhamnose
D(−) ribose
D(+) xylose
meso-inositol
β-D(−) fructose
β-D(+) mannose
D(+) galactose
α-D- glucose
D(+) trehalose
β-D-glucose
sucrose
D(+) mannitol
dulcitol

The standards were run separately and as a mixture under identical conditions as the experimentals. Retention times for the samples and standard sugars were recorded and comparisons made for identification of the sugars.

RESULTS AND DISCUSSION

The color of most of the fruit fly crop contents used in this study was amber. However, the crop content color may vary depending on the collecting site and season. For example, the crop contents have been observed to be colorless, brown, black, purple, and yellow, in addition to the more common amber color in a study of the seasonal distribution of color in crop contents of *Dacus dorsalis* found in guava groves at four locations on Oahu during 1965 (Nishida, 1965). Also, the crop contents may be opaque in addition to appearing clear. In Nishida's study, the most striking color change occurred during the winter months in fruit flies collected exclusively from the Kailua-Waimanalo area where the dominant color was purple (see Table 2). This suggests that a correlation between crop contents and the type of food source found at a particular location may exist. If this is so, then the composition of carbohydrates in the crop contents from fruit flies caught at different locations may also differ, perhaps mirroring the sugar composition of its food source.

The sugar composition of crop contents from fruit flies and of fruits collected at the various locations is shown in Table 1. A typical gas-liquid chromatographic analysis of a sample from Lyon Arboretum and a standard TMS-sugar profile are shown in Fig. 2. (Peaks not identified were designated unknown sugars *a, b, c*...etc.)

Fruit flies collected from all three field locations and even those from the USDA Fruit Fly Investigations Laboratory in Manoa (where the flies are reportedly maintained on sucrose cubes and protein hydrolysate) contained 7 sugars in common. These were fructose, mannose, galactose, mannitol, glucose, sucrose, and unknown sugar *a*. In addition, all fruits analyzed contained these same sugars and sugar alcohols. The very small trehalose peak which appears in the analyses may be attributable to minor blood sugar contamination.

The most distinctive crop sugar complement was found in fruit flies collected at the Lyon Arboretum, containing unknown sugars *g, h, k,* and *l* not found in flies captured at the other locations nor in the fruits analyzed.

Glycerol was detected in both surinam cherry and guava, but fruit flies collected near surinam cherry trees at Lyon Arboretum lacked this compound in their crops. However, flies collected at Waimanalo and Mt. Tantalus, where guava is found, contain this compound.

Unknown sugar *m* was present in all flies except those collected at Waimanalo. Only pomegranate and mango contained this unknown sugar.

Methyl eugenol was detected in flies from Mt. Tantalus and Lyon Arboretum only, which was not unexpected since the flies were seen to be feeding on the attractant during their capture. As expected, no methyl eugenol was detected in the fruits analyzed nor in the flies obtained from the USDA.

Only fruit flies from Mt. Tantalus, Waimanalo, and the USDA contain unknown sugars *b, f,* and *e* respectively. The fruits analyzed all lacked these sugars.

During April, 1976, ripening mango fruits were uncommon, but one was obtained for sugar analysis. It proved to have a most unique complement of sugars- dulcitol and inositol - not found in any flies analyzed at that time. It is anticipated that as more mangoes ripen and become a common food source for fruit flies, crop contents may well reflect these sugar alcohols.

If any similarities are to be established for correlation purposes, they should appear in the unknown sugar group, which would represent in all probability in this study the less common or more unique sugar complement. In the analyses, it appeared that at most only two unknown sugars were shared by a fruit and fruit flies collected from a common site (guava and fruit flies collected from Mt. Tantalus in respect to unknown sugars *a* and *m*). Papaya showed correlation with flies from Waimanalo by the presence in both of unknown sugar *c*. One of us (J.M.T.) observed *Dacus dorsalis* feeding on papaya in Waimanalo near the collecting site. Pomegranate shared unknown sugar *m* with fruit flies from Mt. Tantalus, while mango showed unknown sugar *d* in common with flies from Waimanalo.

A brief survey of the literature indicates that some sugars, such as the unknown sugar complement seen only in the fruit flies captured at the Lyon Arboretum, may originate from coccid and/or aphid honeydew. Bateman (1972) suggested that, rather than fruits, insect honeydew may well be the principal source of food for adult fruit flies in nature. In support of this is the work of Neilson et al. (1965) on the apple maggot, of Steiner (1955) on *Dacus* in Hawaii and Varley (1947) on the knapweed gall fly, who found these insects to feed on insect honeydew. Further, Hagen (1958) believed that honeydew excreted by homopterous insects is nutritionally complex, and that tephritids probably seek honeydew as a natural food and perhaps even require it.

In the few studies that have been done on analysis of honeydew, the presence of sucrose, fructose, and glucose in addition to other less common mono-, di-, and trisaccharides has been demonstrated. Gray (1952) showed these 3 common sugars to be present in pineapple mealybug honeydew, while Gray and Fraenkel (1954) detected the disaccharide fructo-maltose and the monosaccharide glucose-1-phosphate in addition to sucrose, fructose, and

TABLE 1. *Sugar profile of crop contents from oriental fruit flies from 4 locations, and of juices from fruits obtained in the vicinity of fly capture sites*[1]

	GLYCEROL	FRUCTOSE	MANNOSE	GALACTOSE	MANNITOL	DULCITOL	α—D—GLUCOSE	β—D—GLUCOSE	INOSITOL (MESO)	SUCROSE	TREHALOSE	METHYL EUGENOL	UNKNOWN *a*	UNKNOWN *b*	UNKNOWN *c*	UNKNOWN *d*	UNKNOWN *e*	UNKNOWN *f*	UNKNOWN *g*	UNKNOWN *h*	UNKNOWN *k*	UNKNOWN *l*	UNKNOWN *m*
Waimanalo	X	X	X	X	X		X	X		X	X		X	X	X	X							
Mt. Tantalus	X	X	X	X	X		X	X		X	X	X	X					X					X
Lyon Arboretum		X	X	X	X		X	X		X	X	X	X						X	X	X	X	X
U.S.D.A.		X	X	X	X		X	X		X	X		X			X	X						X
Guava	X	X	X				X	X		X			X										X
Surinam cherry	X	X	X	X	X		X	X		X			X										
Mango		X	X	X	X	X	X	X	X	X			X			X							X
Pomegranate		X	X	X	X		X	X		X			X										X
Papaya		X	X	X	X		X	X		X			X		X								

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[1] The symbol X represents the presence of a peak on the gas chromatogram (from a sample site) corresponding to the retention time of a standard TMS-sugar. Peaks not corresponding to known sugar standards are designated *a,b,c*....etc. See text for further details.

TABLE 2. *Seasonal distribution of crop content color of* Dacus dorsalis.[1]

Waimanalo

Color	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Brown	2	2	0	0	0	0	1	0	0	0	1	0
Amber	11	13	14	12	15	14	12	11	11	7	7	0
Clear	1	0	0	0	0	1	2	4	4	0	0	2
Black	-	0	1	0	0	0	0	0	0	0	0	0
Purple	1	0	0	0	0	0	0	0	0	8	7	13
Yellow	0	0	0	0	0	0	0	0	0	0	0	0

Kailua

Color	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Brown	1	0	0	2	1	3	0	0	1	0	0	0
amber	13	12	12	12	13	12	12	10	11	4	11	11
Clear	0	0	0	1	0	0	3	5	3	0	3	0
Black	1	3	3	0	0	0	0	0	0	0	0	1
Purple	0	0	0	0	0	0	0	0	0	10	1	3
Yellow	0	0	0	0	0	0	0	0	0	0	0	0

FIG. 1. Full crop in a male oriental fruit fly adult is shown as a globose bead between the two parts of the fly body.

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[1] Samples of 15 males were collected at monthly intervals in guava groves at Waimanalo and Kailua, Oahu during 1965 (Nishida, in press).

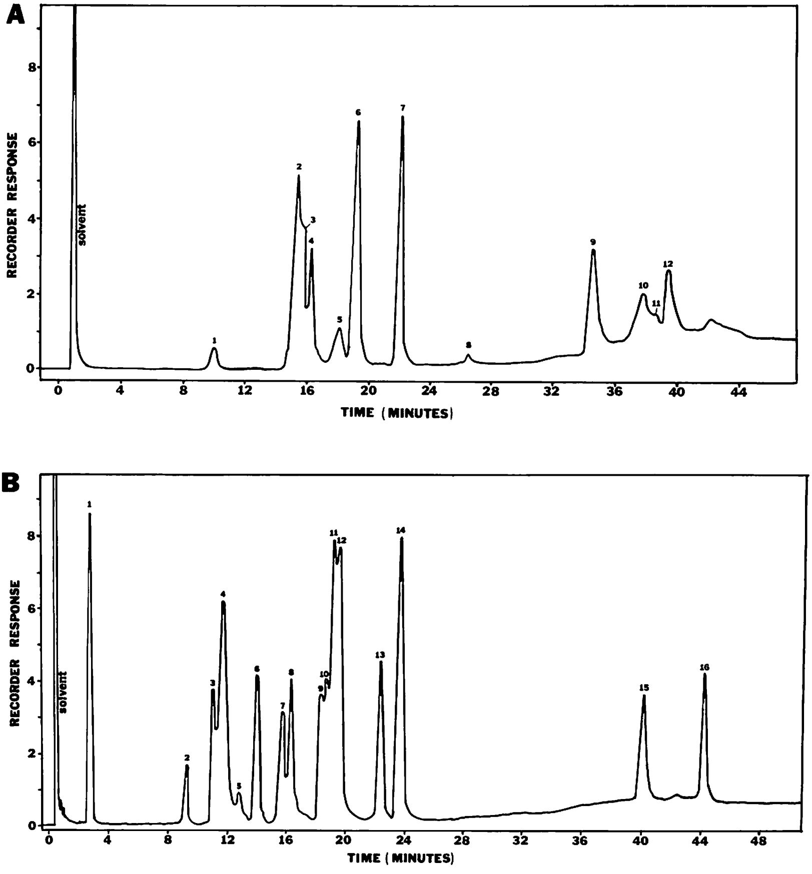

FIG. 2(A) Gas-liquid chromatogram of TMS-derivatized sugars from the pooled crop contents of 50 oriental fruit flies captured at Lyon Arboretum, Oahu, Hawaii. Peaks were identified by comparing retention times with a standard TMS sugar mixture. Peaks not identified were designated *a, b, c*...etc.

Peaks:				
	1	methyl eugenol	7	β-D-glucose
	2	fructose	8	unknown sugar *l*
	3	unknown sugar *a*	9	unknown sugar *g*
	4	mannose	10	unknown sugar *h*
	5	galatose	11	unknown sugar *k*
	6	α-D-glucose	12	sucrose

(B) Gas-liquid chromatogram of a standard TMS-sugar mixture and methyl eugenol in hexane programmed for separation between 100°C to 220°C at 2°C/min with a 16 minute hold. Further details on operating parameters in the text.

Peaks:				
	1	glycerol	9	D(+)galactose
	2	methyl eugenol	10	(+)mannitol
	3	L(+)arabinose	11	dulcitol
	4	D(−)ribose	12	α-D(+)glucose
	5	L(+)rhamnose	13	β-D(+)glucose
	7	β-D(−)fructose	15	sucrose
	8	β-D(+)mannose	16	D(+)trehalose

glucose. Wolf and Ewart (1955) found trisaccharides in the honeydew of two scale insects, while Hackman and Trikojus (1952) detected the sugar alcohol ribitol in Australian *Ceroplastes*. All of the above sugars can be used as is or hydrolyzed to simpler sugars in sustaining fruit flies.

From the results obtained in this study, it is concluded that the sugar profiles of fruit fly crop contents differ, in respect to one or more sugars, between flies from different locations. The correlation between crop sugars and food source is less clear. It is obvious that a more comprehensive analysis of additional fruits is necessary to establish whether a definite correlation exists. Because honeydew may be a primary source of food for fruit flies, a study is presently underway to analyze the honeydew from insects feeding upon selected plants in the vicinity of the fruit fly collection sites.

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