NECTAR-FEEDING HABITS OF *AEDES TAENIORHYNCHUS*[1]

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ABSTRACT. *Aedes taeniorhynchus* were collected on the wing at several times during the day and night, in Indian River and Brevard County, Florida. Nonblooded females were individually analyzed for fructose and glycogen. Fructose content of mosquitoes collected early in the morning and held without food during the day diminished to approximately the same level as in mosquitoes collected late in the afternoon. However, fructose content of mosquitoes collected before sunset and held without food overnight diminished greatly, whereas that of mosquitoes collected in the morning always exceeded that of evening collections. Maximum fructose content occurred within one or 2 hours after sunset. Apparently, these mosquitoes take little or no nectar during the day, and feed soon after the onset of darkness.

INTRODUCTION

The importance of nectar feeding by mosquitoes has been recognized for some time. The opportunity to bite a distant host is largely dependent on the mosquito's ability to find nectar to provide flight energy. *Aedes taeniorhynchus* (Wiedemann) had been observed feeding on flower nectars (Haeger 1955), but no positive identification of crop content was attempted at that time. Since only mosquitoes visiting flowers were observed, this early investigation gave no indication of the frequency of nectar feeding by the total population. Furthermore, if feeding took place after sunset, observations would be difficult.

The development of a simple method to detect fructose (Van Handel 1972) as a specific marker for nectar (and honeydew) made it possible for the first time to survey the nectar-feeding frequency of mosquito populations. Using this method, Bidlingmayer and Hem (1973) surveyed populations of 24 mosquito species in Florida, collected by suction trap and power aspirator. Among the most prominent nectar feeders was *Ae. taeniorhynchus*, the main pest mosquito of the Florida salt marshes. During mid-summer, 2 of every 3 specimens contained nectar. These observations, and many others, were only qualitative, and gave no indications of feeding periodicity. In the present work by measuring fructose and glycogen content quantitatively, we were able to pinpoint the time of nectar feeding in the field.

MATERIALS AND METHODS

A hand-held power aspirator was used to sample *Ae. taeniorhynchus* attracted to the collector at 2 locations, one in the immediate vicinity of the Florida Medical Entomology Laboratory (FMEL hammock) and one at Sebastian Inlet State Park (Brevard County). Collections were frozen immediately and analyzed within 24 hours. When mosquitoes were observed in the laboratory, they were held in a 1,000-liter cage and provided with water.

For nutritional analysis (Van Handel 1985), individual mosquitoes were placed in culture tubes and crushed in 0.2-ml 2% sodium sulfate solution. This solution was mixed with 0.5-ml methanol and centrifuged. The supernatant, containing the sugars, was transferred to a different culture tube and evaporated to about 0.2-ml. Anthrone solution was added to a 5-ml mark and the reaction allowed to proceed at room temperature (25°C) for 45–60 min. The standard was a 50-μg sucrose (50-μl 0.1% solution), in the anthrone test equivalent to 25-μg fructose. After reading the optical density, these same supernatants and the sucrose standards were heated for 12 min at 90°C to obtain the value for total sugars.

To the precipitates, containing the glycogen, anthrone solution was added also to a 5-ml mark, and the tubes heated for 12–15 min at 90°C. We used 50-μg glucose (50-μl 0.1% solution) as standards for glycogen (Van Handel 1985). Each mosquito was analyzed for fructose, total sugars and glycogen. Mosquitoes with an optical density below 0.04 (less than 2-μg fructose) in the cold anthrone reaction were considered to be negative for nectar. When mosquitoes were analyzed for fructose only, they were crushed in 5-ml anthrone solution and assayed in 45–60 min. Data in the tables represent the means of 12 mosquitoes.

RESULTS

Comparison between morning and evening collections: Aedes taeniorhynchus from the FMEL hammock were collected each morning at 0800 h, and analyzed immediately or held until 1600

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h without access to nectar. At that time, a new field collection was made and analyzed immediately, or held until 0800 h the following morning without access to nectar. In order to minimize variation, 10 collections were made consecutively from Monday, August 14, to Friday, August 18, 1989. Mosquitoes from the 0800-h collections always contained more fructose than those from the 1600-h collections. Apparently, the main nectar feeding took place between 1600 and 0800 h. To evaluate whether daytime nectar feeding took place, mosquitoes from an 0800-h collection were held in a large (1,000-liter) cage with water only, until 1600 h. At that time, a second field collection was made. Obviously, without access to nectar the fructose content of these caged mosquitoes diminished. However, it diminished no more than in mosquitoes freshly collected from the field. The overall average fructose content of all field collections at 1600 h was 6 μg, the same as for the 0800-h collections held without food until 1600 h. This strongly suggests that in the field no nectar feeding had taken place from 0800 to 1600 h. When specimens collected at 1600 h were compared with those collected at 0800 h the next morning, the fructose content was consistently higher in morning mosquitoes. In contrast, specimens collected at 1600 h and held without food until the next morning contained very little nectar (Table 1). The overall average fructose content for mosquitoes collected in the morning was 13 μg, whereas those held without food from 1600 until 0800 h contained only 0.5 μg. These data suggest again that in the field main nectar feeding took place between 1600 and 0800 h.

The total sugar content, as measured by hot anthrone, included in addition to the marker fructose other nectar sugars (mostly free and sucrose-bound glucose) and the hemolymph sugar trehalose. It is therefore understandable that total sugar content correlated well with the fructose content (Table 1), and that the conclusion drawn from total sugars is consistent with that from fructose. In the absence of fructose the total sugar content approaches that of trehalose.

Glycogen is synthesized and accumulates as a result of nectar feeding, after a delay of several hours. It is therefore not surprising that the lowest and highest values for fructose corresponded with glycogen content, and that even during short-term food deprivation not only sugars, but also glycogen, were used as a respiratory substrate (Table 1).

Carbohydrate content during the day and night: To obtain more detailed information on nectar feeding, and on the reproducibility of feeding observations, biting collections were made at the Brevard County site, about 40 km north of the FMEL. Collections were made at sunrise, just before sunset, at noon and at midnight (Table 2). Fructose (and total sugar) content was consistently higher at sunrise than at sunset. At midnight peak levels of fructose had already been reached. At noon there was a slight

Table 1. Carbohydrate content (μg/female ± SE) of *Aedes taeniorhynchus*, collected from the FMEL hammock at 0800 h and held without food to 1600 h or collected at 1600 h and held without food to 0800 h.

Date (1989)	Time (h)	Fructose	(%) positive	Sugars	Glycogen
Aug. 14	0800	6 ± 2.5	(60)	30 ± 5	31 ± 4
Same, caged	0800–1600	5 ± 2	(33)	26 ± 2	22 ± 2
Aug. 14	1600	4 ± 1	(33)	23 ± 2	21 ± 2
Same, caged	1600–0800	1 ± 1	(16)	19 ± 2.5	17 ± 3
Aug. 15	0800	16 ± 5	(85)	43 ± 10	32 ± 4
Same, caged	0800–1600	11 ± 2.5	(66)	34 ± 7	34 ± 5
Aug. 15	1600	7 ± 6	(33)	30 ± 9	42 ± 4
Same, caged	1600–0800	1 ± 1	(8)	16 ± 4	17 ± 3
Aug. 16	0800	6 ± 1	(50)	20 ± 2	16 ± 3
Same, caged	0800–1600	2 ± 1	(25)	21 ± 2	21 ± 3
Aug. 16	1600	1 ± 1	(16)	15 ± 1	30 ± 8
Same, caged	1600–0800	0	(0)	9 ± 2	13 ± 4
Aug. 17	0800	16 ± 6	(85)	40 ± 8	83 ± 9
Same, caged	0800–1600	5 ± 3	(33)	23 ± 4	42 ± 6
Aug. 17	1600	4 ± 4	(10)	22 ± 2	60 ± 9
Same, caged	1600–0800	0	(0)	10 ± 4	13 ± 2
Aug. 18	0800	27 ± 7	(8)	73 ± 15	75 ± 9
Same, caged	0800–1600	8 ± 3	(33)	30 ± 5	33 ± 5
Aug. 18	1600	14 ± 3	(56)	60 ± 10	45 ± 5
Mean Aug. 14–18					
Field-collected (n = 60)	0800	13		41	47
Same, caged until (n = 60)	1600	6		27	30
Field-collected (n = 60)	1600	6		30	40
Same, caged until (n = 48)	0800	0.5		13	15

Table 2. Carbohydrate content (μg/female ± SE) of *Aedes taeniorhynchus* collected at the Brevard County site through day and night.

Date (1989)	Time (h)	Fructose	Total sugars	Glycogen
Jul. 26	0600	14 ± 4	55 ± 6	28 ± 4
Jul. 26	Noon	10 ± 3	48 ± 4	34 ± 3
Jul. 26	2000	2 ± 0.5	30 ± 2	39 ± 4
Jul. 27	0600	27 ± 4	57 ± 8	43 ± 5
Jul. 27	2000	18 ± 5	50 ± 10	36 ± 4
Aug. 1	1900	13 ± 2	36 ± 6	32 ± 3
Aug. 1	Midnight	34 ± 10	70 ± 2	35 ± 4
Aug. 2	0700	32 ± 5	64 ± 10	50 ± 5
Aug. 2	1900	5 ± 2	30 ± 5	30 ± 4
Aug. 2	Midnight	35 ± 6	88 ± 15	28 ± 4
Aug. 3	0700	40 ± 8	95 ± 17	31 ± 3

decline in fructose compared with the morning samples. Fluctuations of glycogen levels were more complex. A high fructose and low glycogen level is consistent with a very recent nectar meal. A new sugar meal drives glycogen to higher levels, followed by a decline of fructose levels. However, flight may drive the glycogen level down again, along with sugar levels. In general, fluctuations of glycogen were rather small during a 24-hour period (Table 2).

Pinpointing the nectar meal: Since all evidence pointed toward a very short feeding period, collections were made between 1800 and 2100 h, from an hour before sunset to an hour after darkness. These data (Table 3) prove that soon after darkness *Ae. taeniorhynchus* fed heavily on nectar and at that time contained maximal amounts of fructose.

Flight activity under laboratory conditions: When utilization of nutrients was studied in the laboratory, it was demonstrated that the mobilization of caloric reserves depended on flight activity and therefore on cage size. Mosquitoes were collected in the field at 0900 h each morning and maintained without sugar in a small (0.4-liter) or a large (1,000-liter) cage. After 24 hours, under natural light conditions, the decline of fructose and glycogen levels was much greater in the large cage (Table 4).

Blood-fed mosquitoes: When mosquitoes were allowed to blood feed, they were found to contain the same carbohydrate levels (65 ± 8 μg for fructose, 120 ± 16 μg for total sugars and 34 ± 4 μg for glycogen) as specimens from the same host seeking collection that did not contain blood. Apparently in *Aedes taeniorhynchus* a high nectar content induced neither abstention from host seeking nor from subsequent blood feeding.

Table 3. Fructose content (μg/female ± SE) of *Aedes taeniorhynchus* collected at the Brevard County site between 1800 and 2100 h.

Date (1989)	1800 h	2100 h
Aug. 7	17 ± 2	32 ± 5
Aug. 19	15 ± 2	60 ± 8
Aug. 20	33 ± 8	64 ± 8
Aug. 22	3 ± 1	64 ± 12
Aug. 23	7 ± 3	42 ± 11

DISCUSSION

We used quantitative assays for the analysis of fructose, total sugars and glycogen. The results strongly support the conclusion that in the field nectar feeding by *Ae. taeniorhynchus* takes place during a short period after sunset, and that little or no nectar feeding occurs during daylight hours. As expected with field experiments and expressed by the standard error of the means, within-sample variability was high. This was especially true when the average value for fructose was low, and was often caused by a few mosquitoes with relatively high fructose content. In order to establish a high degree of reliability, we made consecutive collections during relatively short time periods. Without exception, collections taken between late afternoon and just before sunset had a lower nectar content than corresponding collections taken between dark and early morning. The periodicity of these results suggest that the population was rather homogeneous and may have represented a single large brood. But, even within a brood, one should expect day-to-day variability due to wind, rain and nectar availability.

Aedes taeniorhynchus is a far ranging, active species. When restricted in its movements by a small cage, it uses far less energy than in a large cage that affords more opportunity for flight (Table 4). Fructose is the most variable of the 3 components studied, whereas glycogen was the most constant and seemed to serve as a buffer to maintain flight capability (Nayar and Van Handel 1971). All mosquitoes in this study were host seeking and sampled as they were attracted to the collector. Freshly blood-fed females had

Table 4. Carbohydrate content (μg/female ± SE) of field-collected *Aedes taeniorhynchus*, maintained for 24 h without food in small and large cages.

Date (1989)	Carbohydrate	Field (baseline)	Small cage	Large cage
Jul. 24	Fructose	23 ± 5	8 ± 3	1.5 ± 1
	Glycogen	37 ± 6	32 ± 6	16 ± 2.5
Jul. 31	Fructose	36 ± 7	9 ± 4	0.5 ± 0.5
	Glycogen	50 ± 8	43 ± 5	23 ± 4
Aug. 2	Fructose	61 ± 10	8 ± 5	3 ± 2
	Glycogen	46 ± 7	32 ± 3	22 ± 3

the same high nectar and glycogen content as nonblooded specimens.

Culex nigripalpus (Theobald) collected on the wing in light and bait traps contained much less sugar and glycogen than specimens collected at resting sites (Day and Van Handel 1988). At resting sites all freshly blood-fed *Cx. nigripalpus* mosquitoes were fructose-negative and contained much less glycogen than those, collected at the same resting site, that had not blood-fed (unpublished data). Low carbohydrate content may be a prerequisite for blood feeding by *Cx. nigripalpus*, but not for *Ae. taeniorhynchus*.

Using a qualitative test for fructose, other workers have investigated nectar feeding times. Magnarelli (1979) found that in *Ae. cantator* (Coq.) and *Ae. sollicitans* (Walker) the percentage of nectar-positive specimens was maximal between 2000 and 2100 h. Reisen et al. (1986) demonstrated that nectar feeding in *Cx. tarsalis* Coq. occurred during the second half of the night and that peak nectar feeding followed maximum host seeking activity. Andersson and Jaenson (1987) investigated nectar feeding in *Culex* mosquitoes collected from flowers in Sweden. Among *Cx. pipiens* Linn. females, 80% were positive, both in gravid and empty specimens. In *Cx. pipiens* and males of *Cx. torrentium* Martini, peak nectar feeding occurred between 2200 and 0400 h. About 50% of *Cx. pipiens* females that were collected in bait traps had fed on nectar shortly before. This contrasts with our finding (Day and Van Handel 1988) that recently blood-fed and bait-trapped *Cx. nigripalpus* were fructose negative.

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