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THE USE OF AN ANTHRONE REAGENT TO DETECT SUGAR MEALS AND THEIR PERSISTENCE IN THE MOSQUITO *AEDES TRISERIATUS* (DIPTERA: CULICIDAE)

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ABSTRACT

Adults of *Aedes triseriatus* were fed water, blood, and 10% pure and mixed solutions of glucose, fructose and sucrose. Adults were tested for fructose by the cold-anthrone test 0, 1, 4, 12, and 24 h after feeding. Water-fed males and females and blood-fed females were anthrone negative. Glucose-fed males were anthrone negative but some glucose-fed females were weakly anthrone positive immediately after feeding. Many adults fed a mixture of glucose, fructose and sucrose were anthrone negative 12 h after feeding and all were anthrone negative after 24 h. The interpretation of negatives in the anthrone test is discussed with respect to the dynamics of nectar feeding, metabolic rates and sampling regimes.

The detection of carbohydrates by the use of an anthrone reagent was introduced by Dreywood (1946). Van Handel (1972) made the technique popular by proposing a simple procedure whereby hundreds of field-caught mosquitoes could be quickly examined to see if they had fed on carbohydrates, on the assumption that most carbohydrate sources used by adult mosquitoes contain fructose. The test has been extensively used to monitor carbohydrate feeding and its periodicity in mosquitoes (Magnarelli 1978, Andersson and Jaenson 1987), blackflies (Hunter 1977, Walsh and Garms 1980), tabanids (Magnarelli et al. 1979, Kniepert 1980) and many other Diptera. The test provides a sensitive indicator of the presence of fructose, usually interpreted as evidence of nectar feeding and, although it can be semi-quantified (Haramis and Foster 1983), most workers use the test as a binary indicator of nectar feeding. Few workers have assessed the time frame over which the test is interpretable.

Here we examine the ability of the cold-anthrone test to detect fructose in sugar-fed mosquitoes during the first 24 h following a carbohydrate meal. We employ a probable scenario – adults that have fed on moderate quantities of a dilute nectar. The high rates of anthrone-negative specimens found in many field studies suggest that such small carbohydrate meals might be quite common in mosquitoes.

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MATERIALS AND METHODS

Mosquitoes came from a laboratory colony of *Aedes triseriatus* Say established from eggs derived from a long-established laboratory colony at the University of Notre Dame. The rearing method was adapted from Parker (1978). The colony was maintained at 23±1°C under a photoperiod of 14L:10D. On each day of pupation, pupae were transferred from larval-rearing pans to styrofoam cups filled to within 1 cm of the rim with deionized water. The cups were placed in standard wire-screen cages (31×31×31 cm). After the onset of emergence, adults were removed every 4 h so that cohorts of known age were available for testing. Male and female adults for testing were held separately in standard cages and allowed access to water *ad libitum* from a glass-wool water wick in the cage and a glass-wool covering on the cage kept moist with tap water. After experimental feeding, adults were held until examination in small cages (2.5×15×15 cm, plastic on 2 sides and the ends, screening on 2 sides) placed screen-side down on wetted paper toweling. Adults rarely flew in these small cages; therefore the trends in carbohydrate content that we observed reflect a situation close to that resulting from resting metabolism.

In order to feed mosquitoes known quantities of fluids, adults were immobilized by vacuum applied to the dorsal thorax through a 20-μl capillary tube. Adults were manipulated at ×16 under a stereoscopic microscope. The labellum was inserted into a 1-μl droplet of water or sugar solution expressed from a 10-μl microsyringe; the labellum was kept in the droplet until the entire droplet had been ingested. All water- and sugar-fed adults were 24±4 h old at the time of feeding. Females 72±4 h old were blood fed to repletion on a human arm.

To assess the rate at which crop emptying (and therefore, presumably, carbohydrate metabolism) occurred, the volume of the contents of the ventral esophageal diverticulum ("crop") was assessed 0, 4, 12, and 24 h after feeding 1 μl of a 10% solution of fructose, glucose and sucrose (1:1:1). Concentrations are °Brix (g solute per 100 g solution) as would be read refractometrically; a 10% sucrose solution is about 0.3 M and 1 μl of such a solution contains about 1.7 J of energy. Mosquitoes were dissected in a physiological saline (Lum 1961) to which a minute quantity of liquid detergent had been added to facilitate wetting. The fluid volume of the crop was scored on an ordinal scale as follows: 0: no fluid; 1: <0.5 μl; 2: 0.5–1.5 μl; 3: >1.5 μl. Volume assessment was proofed on similarly treated, non-experimental mosquitoes by extracting the crop contents into a 2-μl capillary tube.

The response of mosquitoes to cold anthrone was assessed according to Van Handel (1972). Adults were crushed in a test tube (10×75 mm) containing a 1:1 mixture of chloroform-methanol to which 1 ml of anthrone reagent was added. Reaction was scored after 1 h at room temperature according to the following ordinal scale: 0=no reaction (i.e. no color change); 1=light-green ring; 2=dark-green ring; 3=light-blue ring; 4=dark-blue ring.

We examined the ability of the cold-anthrone test to detect a sugar meal in 1-d-old adults of both sexes fed water or 10% solutions of fructose, glucose, sucrose, and a 1:1:1 mixture of the three sugars, as well as 3-d-old virgin females blood-fed to repletion. Adults were tested immediately after feeding. We also examined the time frame over which sugar meals could be detected by examining at 1, 4, 12 and 24 h after feeding, mosquitoes fed the 3-sugar mixture at 1 d of age.

An exact 2-tailed Mann-Whitney U test (Mehta and Patel 1992) was used to compare crop-fluid and reaction-intensity ranks. The maximal probability of a type-1 error was set at 0.05.

Table 1. Crop volume of *Aedes triseriatus*

Condition[b]	Time (h)[c]	Sex	Ranked volume of crop contents[a] 0	1	2	3	N
Unfed	0	♂	15	0	0	0	15
Unfed	0	♀	15	0	0	0	15
Sugar-fed[d]	0	♂	0	0	60	0	60
Sugar-fed	0	♀	0	0	60	0	60
Sugar-fed	1	♂	0	0	14	1	15
Sugar-fed	1	♀	2	3	9	0	14
Sugar-fed	4	♂	1	1	13	0	15
Sugar-fed	4	♀	1	2	12	0	15
Sugar-fed	12	♂	8	13	0	0	21
Sugar-fed	12	♀	2	13	0	0	15
Sugar-fed	24	♂	9	6	0	0	15
Sugar-fed	24	♀	8	7	0	0	15

[a]0: no fluid; 1: <0.5 μl; 2: 0.5–1.5 μl; 3: >1.5 μl.
[b]All adults were 24 ± 4 h old at the beginning of the experiment.
[c]Time between feeding and dissection.
[d]1 μl of a 10% glucose-fructose-sucrose mixture (1:1:1).

RESULTS

Unfed adults had empty crops (Table 1). Judging by the crop volumes, all or nearly all of the fluid solutions fed by pipette were diverted to the crop – most adults had rank-2 crop volumes immediately after feeding (Table 1). Crop emptying was detectable in some females as early as 1 h after feeding and in males as early as 4 h after feeding; the sex-dependent difference in crop volumes at 1 h post-feeding was highly significant ($U=147$, $p=0.0105$) but in view of the patterns at 4 and 12 h, we interpret this only as evidence of high variance in the crop-emptying process. By 24 h, crops of all males and females were empty or nearly so (Table 1). As expected, water-fed males and females, and blood-fed females of *Ae. triseriatus* did not react with the anthrone reagent (Table 2). Glucose-fed males were anthrone-negative at hour 0 but, unexpectedly, a large proportion of glucose-fed females was weakly anthrone positive at hour 0 (Table 2). A positive anthrone response to 10 and 27% glucose but not to 3.3% glucose was confirmed in vitro. All adults fed fructose, sucrose, or the 3-sugar mixture exhibited strong responses at time 0 (Table 2). There were no other sex-related differences in anthrone responses ($p>0.05$).

All adults tested at 1 and 4 h were strongly anthrone positive (Table 2). However, by 12 h after feeding, many males and some females were anthrone negative (Table 2); the difference between the sexes at 12 h may be significant ($U=88.5$, $p=0.021$) but is more likely to reflect only high variance in crop emptying. By 24 h after feeding, all adults were anthrone negative even though small numbers of adults at this time still had small quantities of fluid in the crop (Table 1).

DISCUSSION

The anthrone test provides a highly sensitive test for fructose (Van Handel 1972). We detected weak anthrone responses to glucose immediately after feeding in females and *in vitro*, but not in males and not at later times.

Table 2. Anthrone response in adults of *Aedes triseriatus*

Treatment[b]	Time (h)[c]	Sex	Anthrone Reaction[a] 0	1	2	3	4	N
Water	0	♂	15	0	0	0	0	15
Water	0	♀	15	0	0	0	0	15
Blood[d]	0	♀	31	0	0	0	0	31
Glucose	0	♂	15	0	0	0	0	15
Glucose	0	♀	6	9	0	0	0	15
Fructose	0	♂	0	0	0	0	15	15
Fructose	0	♀	0	0	0	0	15	15
Sucrose	0	♂	0	0	0	1	14	15
Sucrose	0	♀	0	0	0	0	15	15
GFS[e]	0	♂	0	0	0	0	15	15
GFS	0	♀	0	0	0	0	15	15
GFS	1	♂	0	0	0	0	15	15
GFS	1	♀	0	0	0	3	12	15
GFS	4	♂	0	0	0	5	10	15
GFS	4	♀	0	0	0	1	14	15
GFS	12	♂	12	5	0	4	0	21
GFS	12	♀	4	1	3	5	2	15
GFS	24	♂	15	0	0	0	0	15
GFS	24	♀	15	0	0	0	0	15

[a]Ordinal scale: 0=no reaction; 4=very strong reaction.
[b]Except for blood-fed females, all adults were 24±4 h old at the time of feeding. All sugar feedings were 1 μl of a 10% solution.
[c]Time between feeding and testing.
[d]Fully engorged on human blood at age 72±4 h.
[e]Glucose-fructose-sucrose mixture (1:1:1).

Inasmuch as no other authors have reported this, the most likely explanation is low-level contamination in a few replicates.

Although it is generally accepted that carbohydrates are essential resources for most species of mosquitoes (Van Handel 1965, Nayar and Sauerman 1971a,b; Nayar and Van Handel 1971; Yuval 1992), many studies that have employed the cold-anthrone test report high proportions of fructose-negative individuals, sometimes even when the adults were collected while nectar feeding (*inter alia*: Harada et al. 1976, Magnarelli 1977, 1978; El-Akad et al. 1989), this in spite of the known high sensitivity of the test. Some reports of low frequencies of fructose-positive adults may reflect true low rates of nectar feeding but there are many reports of apparently low frequencies of fructose-positive adults in temperate-zone populations that are well known to feed extensively on flower nectar (Sandholm and Price 1962, Grimstad and DeFoliart 1974, 1975). The issue addressed here, then, is a resolution of this paradox – an established critical need for carbohydrates in many blood-sucking Diptera yet high frequencies of nectar-negative adults in the field.

Few workers who have used the anthrone test have attempted to assess the interpretability of anthrone-negative specimens. Although it seems widely recognized (e.g. Magnarelli 1978, Haramis and Foster 1983) that the test provides evidence of *recent* nectar feeding, the time frame over which the test is interpretable is rarely quantified, in spite of the fact that nectar-acquisition and metabolic rates are likely to be strongly dependent on species, sex, habitat, and season. We know of only two studies (Reisen et al. 1986, Andersson and Jaenson 1987) in which the temporal applicability of the anthrone test was measured. In an insectary study, Reisen et al. (1986) showed that fructo-

se-fed *Culex tarsalis* Coquillett could become anthrone negative 48 h after feeding on fructose and almost all adults were anthrone negative after 72 h. Field-collected mosquitoes became anthrone negative much more rapidly; few *Cx. tarsalis* adults collected in red boxes in the afternoon were fructose positive whereas most adults collected in the early morning were fructose positive (Reisen et al. 1986). In a field study of *Culex* mosquitoes, Andersson and Jaenson (1987) showed that adults captured while nectar feeding were fructose negative 20 h later. If nectar-fed adults become anthrone negative over an interval of time that is less than the period length of the nectar-feeding behaviors (e.g. Andersson and Jaenson 1987) then serious underestimates of the frequency of nectar feeding in populations could result, artefacts attributable to the 3-way interaction of the sampling regimen with the nectar-feeding periodicity and metabolic rates in the insects. If, on the other hand, nectar meals are large and require >24 h for digestion (e.g. Cupp and Collins 1979) or if nectar meals are taken at intervals shorter than the digestion period (Walsh and Garms 1980) then the anthrone test cannot be used to assess diel periodicities of nectar feeding. These periodicities and metabolic rates, and their sexual, diel and seasonal variances are not known for most natural populations; if these variables are not quantified, then we caution that the anthrone test has little utility beyond showing that *some* adults in the population have fed on fructose-containing material at *some* time in the recent past.

We have shown that nectar-fed adults of *Ae. triseriatus* can become anthrone negative in as little as 12 hours after feeding. In our study, the adults were confined to small cages in which sustained flight was difficult. In a natural situation in which adults must fly to find nectar and blood sources, and resting, mating and oviposition sites, the metabolic rates can be expected to be much higher (Reisen et al. 1986). If, as is likely in many habitats, nectar is in short supply and the periodicities of nectar feeding by and metabolic rates of various segments of the population are not known, then the interpretation of anthrone-negative readings will be very challenging indeed; the negatives could as likely be due to sampling artefacts as to real differences in nectar-acquisition rates. To the extent that this interpretation is valid, it is difficult to evaluate the many papers that have explored nectar feeding by use of the anthrone test.

Nectar-feeding in many species is now well established but the details and dynamics of nectar foraging in most species are only rudimentarily or not at all known. If the data derived from the anthrone test were combined with estimates of carbohydrate-ingestion and metabolic rates, along with their diel and seasonal variances, then the anthrone test could be much more valuable in the interpretation of the dynamics of field populations. Perhaps it is time to abandon reliance on a binary test whose time frame is likely to be highly dynamic. From an energetics point of view, much more is to be gained from quantifying the energy contents of insect crops (e.g. Haramis and Foster 1983, Smith and Gadawski 1994, Smith et al. 1994).

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