ators were notified of *Ae. aegypti* reinfestation. Their cooperation was solicited in any action taken by the Health Department with eradication procedures.

b) Statutory requirements relating to aircraft disinsectization were relayed to those concerned; however, the difficulties encountered with the practical application of aircraft disinsectization in a thorough manner precluded this as an effective control measure.

c) At the earliest opportunity the freight shed was closed and the interior fogged with ULV-applied pyrethrum. The interior walls were also sprayed with a residual insecticide (Diazinon).

d) Instructions were given to the Customs Department to ensure that the interiors of all freight containers arriving from south of Bermuda were treated by Customs Officers with an aerosol pyrethrum spray prior to unpacking.

e) The waste metal dump (land reclamation project) adjacent to the airport was treated as soon as weather conditions were satisfactory with malathion from a thermal fogger.

f) A general cleanup and elimination of possible breeding sites at the airport was undertaken. The results of this operation were not entirely satisfactory despite the importance attached to this aspect of mosquito control.

g) Information was released to the news media concerning the *Ae. aegypti* reappearance at the airport. This resulted in the publication of much general and specific information on the need for effective mosquito control and public awareness of the health and nuisance problems associated with these insects.

No further evidence of *Ae. aegypti* was found until one positive paddle was identified with a light infestation of *Ae. aegypti* eggs on October 6, 1982. This paddle was taken from an ovitrap set in ornamental shrubbery in a partially enclosed passengers walkway facing the runways. A search of the area revealed no further evidence of infestation and the area was fogged several times with pyrethrum from the ULV equipment.

Aedes aegypti has not been found since October 6, 1982. Surveillance activities against reinfestation have been strengthened in view of the permanent threat of the accidental importation of this disease vector from infested countries to the south of Bermuda. The reestablishment of *Ae. aegypti* in Bermuda with a high index infestation would undoubtedly pose a serious threat to public health and Bermuda's reputation as a healthy and popular tourist resort.

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VISUAL QUANTIFICATION OF SUGAR IN MOSQUITOES USING ANTHRONE REAGENT

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The cold anthrone test for fructose (Van Handel 1967, 1968) was developed by Van Handel (1972) for detecting the presence of nectar in the crops of mosquitoes. It is able to detect recent sugar feeding because fructose is invariably present in nectars and fruits, either by itself (in approximately equal proportions with glucose) or in the disaccharide sucrose (Percival 1961, Van Handel et al. 1972). Furthermore, fructose is not present in unfed mosquitoes and is rapidly broken down during nectar meal digestion. Other workers have used anthrone to test for the presence or absence of nectar in mosquitoes (for example, Bidlingmayer and Hem 1973, Magnarelli 1980), but there appear to have been no studies in which the amount of fructose in field-caught mosquitoes was quantified. During an investigation of nectar feeding by *Aedes triseriatus* (Say) we wanted to roughly measure the amount of nectar present in the mosquitoes, yet avoid the time consuming use of a spectrophotometer. Consequently, we followed the suggestion of Van Handel (1972) of comparing the color reaction of unknowns (field-collected mosquitoes) to a series of standards prepared with known amounts of fructose. However, there appeared to be no published data on the efficacy of the anthrone test, i.e., the precision with which the observed color (or absorbance) indicated the actual amount of fructose present. The primary objective of this experiment was to test the reliability of visual quantification of nectar sugars when using anthrone, by feeding

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mosquitoes measured amounts of sucrose from a pipette. We then compared the results of anthrone tests of the mosquitoes with color standards prepared simultaneously with known amounts of sugar.

Sucrose solutions corresponding to 1, 2, 4, 8, 16, 32, 64, 128, 256, and 512 $\mu g/\mu l$ (0.1 to 51.2% solutions) were prepared by: 1) dissolving 51.2 g of reagent grade sucrose in 60 ml of distilled water, and adding water to make 100 ml of solution; and 2) making 9 two-fold serial dilutions. The resulting solutions were stored at −40° C until needed. Sucrose, rather than fructose, was used because it is a 1 : 1 combination of glucose and fructose, which more closely resembles the composition of natural plant sugar sources (Percival 1961, Van Handel et al. 1972). Thus the fructose content of the solutions was exactly half the total sugar content.

Newly emerged (4 days) female *Ae. triseriatus* that had access to water, but no sugar, were used in the experiment. Twenty-four hr before feeding, the mosquitoes were denied access to water so that they would readily imbibe sucrose solution from a pipette. The mosquitoes were individually inserted into vials and anesthetized by chilling the vials on ice. One μl of sucrose solution was drawn into a micropipette (5 μl Yankee® Micropet, Clay-Adams Inc.) with an aspirator. Excess solution was withdrawn by tapping the end of the pipette on filter paper. Each mosquito was picked up by the thorax with a vacuum pen (hollow needle attached to a vacuum), and her proboscis inserted into the lumen of the micropipette. Mosquitoes that failed to imbibe the entire aliquot were discarded. Three mosquitoes were fed each solution. The mosquitoes were immediately inserted into vials and frozen at −40° C.

To test for fructose with anthrone, the mosquitoes were rapidly thawed and dropped into 5 ml test tubes immersed in an ice water bath. Each mosquito was moistened with 2 drops of 1:1 chloroform-methanol solution to remove cuticular wax. After 20 min, each mosquito was gently crushed with a glass rod that was rinsed in methanol between crushings. One-half ml of anthrone solution (Van Handel 1972) was added to each tube. The tubes were agitated on a vortex mixer, then held in a water bath at 26°C for 1 hr. The tubes were agitated again both half-way through this period and at its end. A series of standards was prepared previously by pipetting 1 μl of each of the stock solutions into a test tube along with a blank tube with no sugar. The tubes containing mosquitoes were given code numbers so that the amount of sucrose actually imbibed could not be determined while the results were being read. Each of the experimental tubes was compared to the color strengths of the standards and was designated according to the standard it most closely resembled.

The quantity of sucrose could be determined accurately for the 64-512 μg range by visual comparison to the standards (Table 1). However, in the 4–32 μg range the quantity was usually underestimated by half (one dilution), and below 4 μg sugar-fed and water-fed mosquitoes were often confused. This inaccuracy at low dilutions may have been a consequence of the quantitatively small difference between two-fold sucrose dilutions at the dilute end of the range. A second explanation is that part of the small quantity of sugar in very dilute solutions was absorbed and metabolized during the short interval between feeding and freezing and thus could not react with the reagent. This drawback may be minor, since mosquitoes containing less than 64 μg of sucrose (or 32 μg of fructose) are probably in the last stages of digesting a nectar meal or have taken a small meal; the crop can hold 1–3 μl of nectar, and flower nectar is generally 25–50% (250–500 $\mu g/\mu l$) sugar (Hocking 1953, Percival 1961). Thus our modification may prove useful in investigations that require processing large numbers of specimens quickly while obtaining greater resolution than that provided by a simple anthrone test for the presence or absence of fructose.

Table 1. Accuracy of visual estimation of sucrose in *Aedes triseriatus* fed known amounts of sugar.

Amount of sucrose (μg) fed to mosquitoes	Estimate of amount of sucrose (μg) in mosquitoes by comparison to standards using anthrone test*		
512	512	512	512
256	256	256	256
128	128	128	128
64	64	64	64
32	16	16	16
16	8	8	8
8	4	4	4
4	1	2	2
2	0	1	0
1	0	0	0
0**	0	1	2

* Three females tested with each amount of sucrose.

** Females fed distilled water.

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THE DOSE-PERSISTENCE RELATIONSHIP OF DEET AGAINST *AEDES AEGYPTI*[1]

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Since its introduction (Gilbert et al. 1957), deet has become the most widely used insect repellent in both the civilian and armed forces inventories. At present there are over 250 different commercial formulations of this compound marketed in the U.S., with concentrations of active ingredient ranging from 2 to 100% (United States Environmental Protection Agency 1980). The formulation of deet currently issued to the armed forces consists of 71.25% deet and 3.75% other isomers in an ethanol base. This concentration was apparently chosen as a compromise between user acceptability and repellent efficacy. A 100% concentration was found to be excessively greasy or sticky at room temperature, and this was presumably lowered to the nearest point of user toleration (Shambaugh and Pratt 1959).

Dose-response methods can be used to determine the correlation between the dose of a repellent and the response it elicits (Rutledge et al. 1976). In particular, a threshold dose may often be determined after which additional increments in the dose of a repellent administered elicit little and eventually no increase in response at all. From a practical standpoint such information is useful in optimizing the cost/benefit ratio of a product or for determining a more acceptable and efficient formulation for the user.

The following experiment was conducted to determine the optimal correlation between persistence and dosage of deet applied on human skin using the U.S. Army standard deet formulation and *Aedes aegypti* (Linn.).

The test method used in these experiments is similar to the ED_{50} arm test reported by Buescher et al. (1982). The flexor region of a test subject's forearm is outlined with five 29 mm diameter circles with a plastic template and a felt tipped pen. A control (ethanol) and four serial dilutions of deet (*N,N*-diethyl-*M*-toluamide, 75% in ethanol, Federal Stock No. 6840-753-4963, Airsol Company Inc., Neodesha, KS) in ethanol were assigned at random to the five test areas. Dosages were calculated in mg/cm^2 using a constant application volume of 0.025 ml and spread evenly within the outlined areas with the tip of a glass rod. The plastic test cage containing 15 mosquitoes was then positioned over the treated areas using Velcro® strips, the slide at the bottom withdrawn, and the number of bites received after 90 seconds was recorded. In subsequent test trials the range of dosages applied was adjusted to estimate the ED_{95} of deet at 0, 1, 2, 3, 4, 5 and 6 hours after the repellent was applied. During the 1–6 hour test interval, four male volunteers conducted normal activities but were not allowed to wash, abrade, or conduct vigorous physical activities that might affect the treated areas. Volunteers were used in all testing.

All tests were conducted with nulliparous *Ae. aegypti* (UCSF strain), 5–15 days of age. Mosquitoes were maintained at 27°C and 75% RH under a 12:12 hr photoperiod incorporating 1 hr of simulated sunrise and 1 hr of simulated sunset. Daytime illumination was held at 30 f.c. Larvae were reared on a diet of Purina Guinea Pig Chow® (ground to 40 mesh), brewer's yeast, and undefatted, desiccated, powdered liver (ratio by weight, 4:4:1). Adult mosquitoes were maintained on 10% sucrose *ad libitum*.

Data were analyzed on a Data General Eclipse 330 computer by the method of probit analysis.

[1] The opinions and assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. Citation of trade names in this report does not constitute an official endorsement or approval of the use of such items.

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