THE EFFECTS OF DIET ON SURVIVAL, INSEMINATION AND OVIPOSITION OF *CULEX NIGRIPALPUS* THEOBALD[1]

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ABSTRACT. *Culex nigripalpus* Theobald, was maintained on different concentrations of sucrose solution to study sugar-feeding behavior and survival, and on 10% sucrose to study blood-feeding behavior, insemination and oviposition. The F_1 females did not become inseminated and consequently did not oviposit.

Sugar-intake during the first week was high at all concentrations, followed by an almost constant lower level daily intake during the next 4 weeks. This regulation of sucrose intake in females was associated with a build up of energy reserves. While male and female mean survival times varied from 2.6 to 3.1 days on distilled water, colonized males lived from 19.8 to 23.7 days and colonized and F_1 females from 37.5 to 53.4 days on the 3 sucrose concentrations. Insemination began 3 days after emergence with 100% insemination by day 9. Only a small percentage of females blood-fed without previously imbibing sugar solution. Insemination, however, was not a prerequisite for blood-feeding. Only a small percentage of blood-fed colonized and wild caught females oviposited, and oviposition was asynchronous, while repeated blood-feeding and oviposition reduced the mean survival time of colonized females.

INTRODUCTION

Culex nigripalpus Theobald has been colonized in the laboratory (Haeger and O'Meara 1970). The importance of sugar-feeding in the laboratory for prolonged survival, flight and increased fecundity in several species of mosquitoes, including F_1 *Cx. nigripalpus,* has been well established (Galun and Fraenkel 1957, Harada et al. 1971, Nayar and Sauerman 1971a, b, 1973, 1975a, b, Nayar and Van Handel 1971, Salama 1967). Because of the medical and veterinary importance of this species in Florida—a vector of St. Louis encephalitis (SLE) virus in humans (Sudia and Chamberlain 1964), of *Plasmodium hermani* the malaria parasite in turkeys (Forrester et al. 1980, Young et al. 1977) and a potential vector of *Dirofilaria immitis* the heart-worm in dogs (Nayar and Sauerman 1975d)—it is necessary to understand its biology and establish in the laboratory its vectoring potential. This paper describes the effects of intake of 3 concentrations of sucrose and repeated blood-feeding on survival and on the rate of insemination and oviposition, factors related to vectoring potential. Comparisons are also made with F_1 adults, where possible.

MATERIALS AND METHODS

Colonized *Cx. nigripalpus* were initially obtained from Mr. James S. Haeger of this Laboratory. At regular intervals, males from an F_1 generation were introduced into the colony to maintain vigor and behavior as close as possible to the wild population (Haeger and O'Meara 1970). Eggs from colony and F_1 generation females were hatched and reared with 200 larvae/pan at 27°C and LD 12:12 (Nayar 1968).

A series of 4 experiments was conducted using both colonized and F_1 *Cx. nigripalpus* and each experiment was repeated at least once. All experiments were performed in a constant-temperature room at 27°C under LD 12:12 and 80% relative humidity.

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In Experiment 1, quantitative sucrose intake was measured with a potometer as described earlier (Nayar and Sauerman 1974). Thirty 12 hr old females were introduced into a cup containing a potometer charged with a known concentration of sucrose solution. Ten cups were used for each concentration (5%, 10%, or 25%) of sucrose solution, 8 cups had 30 females/cup, while 2 cups were without mosquitoes as controls to determine any loss from evaporation. Four of the 8 cups were monitored daily for the mortality and amount of liquid intake by the females. The remaining 4 cups were used for chemical analyses. So every third day, 12 females were removed, preserved, and then analyzed *in toto* for sugars, glycogen and triglycerides (lipids) by the method of Van Handel (1965). Potometers and cups were renewed every third day.

In Experiment 2, 150 males and 150 females 12 hr old were maintained together per cage (45 cm^3) on different concentrations of sucrose solution (5%, 10%, and 25%) and a distilled water control provided ad lib. to determine their mean survival times (Nayar and Sauerman 1971a). Daily mortality was recorded.

In Experiment 3, a group of 5 cages (45 cm^3) each containing 100 females and another group of 5 cages each containing 100 males and 100 females were maintained either on 10% sucrose solution or distilled water. Thereafter, every day starting at 24 hr a cage from each group was allowed access to a tethered chick for 2 hr at mid-afternoon of the LD 12:12 cycle. Blood-fed females were counted and both the engorged and unengorged females from the cages containing males were examined for the presence of sperm in their spermathecae to determine the rate of insemination.

In Experiment 4, 3 groups of 150 males and 150 females/cage (45 cm^3) were maintained on 10% sucrose solution after emergence. One group was allowed its first blood meal on day 2 post-emergence, another on day 5 and the third on day 7. Each group was allowed access to a tethered chick for 6 hr from mid-afternoon to the end of the light-phase. Thereafter, all groups were allowed to blood-feed on Tuesday and Friday, and allowed to lay eggs over a 12 hr period during the dark phase of the LD 12:12 cycle in a bowl of hay infusion on Monday and Thursday for a period of 8 weeks. Daily mortality, the number blood-fed, and the number of eggs laid were all monitored to determine the rate of oviposition.

Finally, wild blood-fed females were collected in a chick-baited lard-can trap to compare their oviposition behavior with colonized females.

RESULTS

A) Effects of the concentration of sucrose solution on intake (Expt. 1):

When females were maintained on different concentrations of sucrose (5%, 10% and 25%), the intake μl/♀/day, was greatest during the first 5 days after emergence on the 5% sucrose solution and least for the same time period on the 25% sucrose solution (Fig. 1A). However, when these values were converted to cal/♀/day, the caloric intake was substantially higher on the 25% sucrose solution than on the other 2 concentrations (Fig. 1B). The volume and the caloric intake declined considerably after the 5th day and remained essentially constant for the next 4 weeks (Fig. 1A, B). The total intake was inversely correlated, i.e., at 5%, the females ingested 78.1 μl/♀, at 10% 46.8 μ/♀, and at 25% 18.8 μl/♀. Total caloric intake, however, was nearly equal at all 3 concentrations, i.e., 15.6 cal/♀ at 5%, 18.7 cal/♀ at 10% and 18.8 cal/♀ at 25% sucrose solution.

At the end of 35 days, there was no distinct difference in the mortality pattern of the females on the 3 concentrations, with approximately 30 percentage dying in each group.

The amount of sugar *in toto* in females fed on the 5% or 10% sucrose solution was very low (0.08–0.33 cal/♀) and dif-

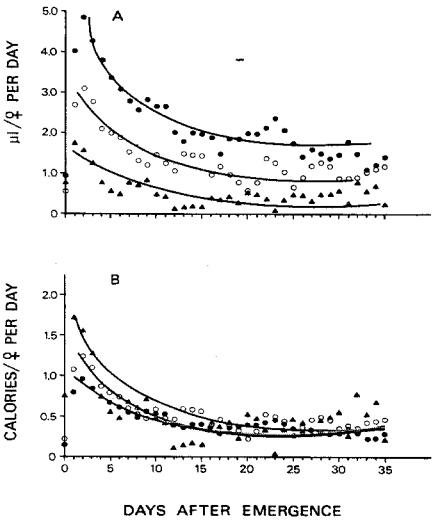

Fig. 1. Three-day running average of daily intake of 3 concentrations of sucrose solution over a period of 35 days after emergence. (A) Volume intake—μl/♀/day. (B) Caloric intake—cal/♀/day. ●———●, 5% sucrose solution; ○———○, 10% sucrose solution; and △———△, 25% sucrose solution.

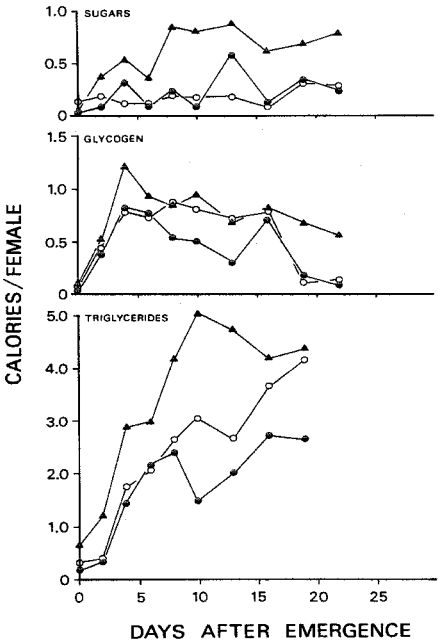

Fig. 2. Energy reserve accumulations at different times after emergence in females fed on 3 concentrations of sucrose solution. ●———●, 5% sucrose solutions; ○———○, 10% sucrose solution; and △———△, 25% sucrose solution.

fered only slightly, suggesting a rapid metabolism of free sugars following ingestion, with apparently little storage (Fig. 2). However, when females ingested a 25% sucrose solution, a larger amount (0.63–0.89 cal/♀) of free sugar was always recovered, especially after the first week (Fig. 2). The level of glycogen stabilized and gradually decreased after the first week in females fed on a 5% and a 10% sucrose solution but was always comparatively high in females fed on a 25% sucrose solution (Fig. 2). Throughout the experimental period the triglyceride level remained consistently lower in females fed on a 5% sucrose solution when compared with those of the other 2 groups, especially in females fed on 25% sucrose solution where the amount remained very high.

The results of experiments with F_1 females were identical and are not presented.

B) Effects of the concentration of sucrose solution on survival (Expt. 2):

A comparison of the mean survival times of colonized and F_1 generation adults maintained on the 3 concentrations of sucrose is given in Table 1. Without sucrose after emergence, there was little difference in the length of survival between colonized and F_1 groups or between the sexes. On a 5% sucrose solution, females of both colonized and F_1 groups had a longer mean survival time than both groups of males. However, on 10% and 25% sucrose solutions, colonized males survived for a much shorter time

Table 1. Comparison of mean survival (days) in colonized and F_1 *Cx. nigripalpus,* 150 males and 150 females per cage were maintained together on different concentrations of sucrose.

Nutrients provided	Colonized Mean survival (days) ♂	Colonized Mean survival (days) ♀	F_1 generation Mean survival (days) ♂	F_1 generation Mean survival (days) ♀
None (distilled water)	2.7 ± 0.05	3.0 ± 0.04	2.6 ± 0.04	3.1 ± 0.05
5% sucrose	23.7 ± 0.9	37.5 ± 1.4	24.7 ± 1.1	38.3 ± 1.4
10% sucrose	23.5 ± 1.2	53.4 ± 1.9	35.7 ± 1.5	53.5 ± 1.9
25% sucrose	19.8 ± 0.9	42.2 ± 1.7	31.8 ± 1.5	50.9 ± 1.6

than the F_1 males, while the difference in the colonized and F_1 female survival times at either concentration was not significant (Table 1).

C) Effects of sucrose- and blood-feeding on insemination (Expt. 3):

A comparison of blood-feeding by both colonized and F_1 females maintained on either distilled water or a 10% sucrose solution revealed that without a prior meal of sucrose, very few females of this species would take blood from a chick when provided ad lib. (Table 2). When they were maintained on a 10% sucrose solution and then provided blood ad lib., more than half of the females had fed by day 3 post-emergence, irrespective of the presence of males (Table 2). On subsequent days, the blood-feeding of females maintained without males showed a decline, whereas blood-feeding remained stable among the females

Table 2. Percentage blood-feeding and rate of insemination in colonized and F_1 female *Cx. nigripalpus,* maintained either with or without males on distilled water and 10% sucrose solution.

Nutrients provided	Days after emergence	Colonized No. of ♀ in cage	Colonized % blood-fed	Colonized % insemination	F_1 generation No. of ♀ in cage	F_1 generation % blood-fed	F_1 generation % insemination
Females only							
Distilled water	1	75	0	0	75	0	0
	2	76	0	0	77	0	0
	3	78	0	0	77	0	0
	4	77	0	0	76	0	0
	5	all dead					
10% sucrose solution	1	75	0	0	76	0	0
	3	80	55.4	0	79	59.2	0
	5	77	37.3	0	81	29.6	0
	7	90	45.3	0	83	30.2	0
	9	81	24.1	0	91	32.0	0
Males & Females							
Distilled water	1	78	0	0	78	0	0
	2	76	0	0	79	0	0
	3	75	0	0	82	0	0
	4	76	0	5	83	3.7	0
	5	All dead					
10% sucrose solution	1	82	6.1	0	78	6.4	0
	3	80	67.5	0	79	69.6	0
	5	100	71.4	50.0	85	71.8	0
	7	85	69.9	67.5	84	82.1	0
	9	98	70.4	100.0	77	67.5	0

maintained with males. Until day 3, none of the colonized or F_1 females maintained with males was found to be inseminated. Insemination of colonized females began after day 3, and 100% of these females were inseminated by day 9. None of the F_1 females was inseminated by day 9, indicating that F_1 females do not readily mate under the conditions described in these experiments.

D) Effects of sugar- and blood-feeding on survival and oviposition (Expt. 4):

Although the F_1 generation *Cx. nigripalpus* females took blood and developed eggs as did the colonized females, they differed by not becoming inseminated or laying eggs. Therefore, no data for F_1 females is given in Table 3.

Between 90% and 100% of females of all 3 groups accepted blood on the day they were first allowed to blood-feed (Table 3). A comparison of the survival and egg laying data of 3 groups of colonized females provided with an initial blood meal at different intervals post emergence revealed no significant effect on their mean survival times (30.2–35.7 days); however, relatively few females oviposited even after several blood meals (Table 3). During the second week only 21 to 32 females laid eggs and in the subsequent 7 weeks, oviposition was varied with many of the females never ovipositing (Table 3).

Wild blood-fed females collected in a chick-baited lard-can trap were separated into: a) 100 females/cage, and b) 200 females/cage and maintained on a 10% sucrose solution. Only 30% and 17% oviposited 5 days after blood-feeding in cages a) and b), respectively. Oviposition sites were provided for 4 more days and approximately 50% of the females never oviposited during the entire experimental period of 9 days.

DISCUSSION

The long term regulation of sucrose intake in colony and F_1 *Cx. nigripalpus* females is similar to that observed in the blowfly *Phormia regina* (Gelperin and Dethier 1967), *Aedes taeniorhynchus* (Nayar and Sauerman 1974), and F_1 *Cx.*

Table 3. Comparison of oviposition by colonized *Cx. nigripalpus* maintained on a 10% sucrose solution and initially blood-fed at different durations after emergence 150 males and 150 females were maintained per cage.

	Treatments*					
	I		II		III	
Durations after emergence	No. ♀ surviving	No. egg-rafts laid	No. ♀ surviving	No. egg-rafts laid	No. ♀ surviving	No. egg-rafts laid
1st week	140		145		145	
2nd week	127	21	123	32	134	30
3rd week	101	7	112	7	121	22
4th week	80	26	96	20	101	30
5th week	51	6	59	4	77	9
6th week	32	3	38	3	46	4
7th week	22	1	22	2	27	3
8th week	10	0	16	1	13	0
9th week	2	0	3	1	8	2
Mean survival	30.2 ± 1.3		32.7 ± 1.4		35.7 ± 1.4	
Total No. (%) egg-rafts		64 (42.7)		70 (46.7)		100 (66.7)

* Treatments I 1st blood meal 2 days after emergence where 90% blood-fed.
II 1st blood meal 5 days after emergence where 95% blood-fed.
III 1st blood meal 7 days after emergence where 100% blood-fed.
Thereafter each group was blood-fed 2 × per week and allowed to lay 2 × per week.

nigripalpus females maintained on a 10% radioactive sucrose solution ad lib. (Nayar et al. 1979). In all of these species, the volume as well as the caloric intake of sucrose increased rapidly after the first day, reached a maximum during the next 5 days, and then declined sharply, stabilizing at maintenance levels with minor fluctuations during the 4 week experimental period. These results agree with the picture of the long-term regulation of sucrose intake observed in other insects (Dadd 1970, Gelperin 1971). Furthermore, the long-term regulation of sucrose intake in *Cx. nigripalpus* is associated with the maximum accumulation of energy reserves (glycogen and triglycerides) at a specific concentration of sucrose as was observed in *Aedes taeniorhynchus* (Nayar and Sauerman 1974).

This study clearly demonstrates that available sucrose is essential for the prolonged survival of both males and females, and is in agreement with earlier studies performed with other species of mosquitoes (Nayar and Sauerman 1971a, 1975a). No distinct differences were observed in the survival of colony and F_1 females, even though colonized male survival was reduced relative to that of F_1 males. Whether this was an effect of colonization, increased activity during mating, or some other factor could not be ascertained by these experiments. However, earlier experiments indicated that larval diet had an effect on energy reserves present at emergence, which in turn affected survival without nourishment after emergence (Nayar and Pierce 1977).

Blood-feeding in both colonized and F_1 females of this species occurred primarily after prior sugar-feeding. This confirmed our earlier observations that unfed 1- to 4-day-old female *Cx. nigripalpus* refused to feed on a chick, even when provided ad lib. up to 12 hr, in both the light and dark phases of a LD 12:12 regime (Nayar and Sauerman 1975b). In this regard *Cx. nigripalpus* differs from many other species of Florida mosquitoes, where females feed freely on a tethered chick without prior sugar-feeding (Nayar and Sauerman 1975b). However, after an initial sugar-feeding, female *Cx. nigripalpus* maintained both with and without males began to blood feed. Blood-feeding rates were stable in females which were inseminated but declined in uninseminated females. Blood-feeding by non-inseminated F_1 females was also studied previously in the laboratory (Edman and Lynn 1975, Edman et al. 1975).

Insemination began 3 days after emergence in colonized females and 100% of these females were inseminated by day 9. Insemination of F_1 females did not occur, however, in the cages used for this laboratory study. The insemination period of the colonized females was slightly longer than that observed in the field where insemination began at about 48 hr post-emergence with 100% being inseminated by the age of 96 hr (Lea and Edman 1972). Similar indications of a delay in insemination in other colonized species were observed by Woodard and Chapman (1977).

A reduction in the mean survival time of females maintained on a sucrose and blood diet as compared to sucrose alone was identical to results observed in *Ae. taeniorhynchus* (Nayar and Sauerman 1971a).

Oviposition by both colonized and wild caught blood-fed females was not synchronized since a few females oviposited each day rather than in a peak. This asynchronous oviposition behavior was observed even though the females blood-fed to repletion and matured about 200 eggs/female (Edman and Lynn 1975, Nayar and Sauerman 1975c). Since only a few of the females oviposited under laboratory conditions, either the conditions for oviposition were not satisfactory or there were other factors not detected in these experiments, which inhibited oviposition.

In conclusion, it is evident that both colonized and F_1 adults behaved in a similar manner, regarding sugar-feeding, blood-feeding and survival. However, insemination and oviposition did not occur among F_1 females in the cages used for

these studies. Colonized females fed repeatedly on blood and oviposited over a period of several weeks.

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BOOM SPRAY APPLICATION OF INSECTICIDE FOR CONTROL OF ESTUARY BREEDING BITING MIDGES (*CULICOIDES*, CERATOPOGONIDAE)

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ABSTRACT. Temephos or Abate® insecticide emulsifiable concentrate formulation applied at the rate of 10% with a salt water carrier and discharged through a telescopic boom from apparatus fitted to a small boat has been 98% effective against *Culicoides molestus* larvae in canal banks. Equipment described enables the application of Abate® E.C. at 0.78 Kg. per ha., and 20 Km. per day are readily achieved in all weathers.

INTRODUCTION

Culicoides molestus (Skuse) has assumed major pest status on the Gold Coast of Queensland, since former wetlands were converted for residential development with artificial canals and white sand beaches. This biting midge species breeds in relatively clean flocculated sand and has effectively colonized man-made breeding places. The larval habitat extends from the 1.5m tideline to the 2m tideline, usually a distance of about 4m on the average slope of canal beaches, and adults emerge at spring tides (new and full moon).

Fox et al. (1968) and Wall and Marganian (1971) showed Abate® insecticide to be effective against *Culicoides* larvae in Puerto Rico and Cape Cod respectively. Abate® insecticide (0,0,0′0′- tetramethyl 0,0′ - thiodi-p-phenylene phosphorothiote) has been used extensively for mosquito control in this area since 1968, with minimal effects to other organisms (Kay et al. 1973), and preliminary trials indicated that it would be effective against *Culicoides* in Queensland.

A rapid practical method was required for the treatment of the 140 Km of canal banks, when the breeding zones were exposed. A boom spray was designed to fit into a small boat and has proved to be highly effective in both distribution of the chemical and reduction in labor costs.

MATERIALS AND METHODS

SPRAYING EQUIPMENT. A 3.6m aluminium boat, driven by 15 horsepower outboard motor, was fitted with a V.D.O.® sumlog and speedometer which accurately measures distance travelled and speed in knots, even at slow speeds.

A 4 horsepower air-cooled 4 stroke petrol engine fitted with a pulley and a vee belt drives a 25mm (1 in) positive displacement gear pump to produce 60 lb. pressure, and a pressure relief valve protects the system. A discharge line allows salt water to be circulated through the pump and discharged back to the sea when the insecticide flow is interrupted.

A pulley and belt fitted on the pump