

Effects of forced egg-retention in *Aedes albopictus* on adult survival and reproduction following application of DEET as an oviposition deterrent

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ABSTRACT: The insect repellent DEET (0.1% concentration), used as a mosquito oviposition deterrent in the laboratory, influenced the retention and maintenance of mature eggs by caged gravid female *Aedes albopictus* Skuse. This egg-retention mechanism could benefit survival because the gravid females were ultimately able to lay maintained eggs upon availability of water, but the length of forced egg-retention time reduced the number of eggs laid per female. Gravid females with retained eggs also laid a higher percentage of eggs that failed to tan, and this percentage increased with time duration of egg-retention. Percent egg hatch was not significantly affected by DEET when used as an oviposition deterrent; however, percent hatch was affected by time duration of egg-retention in both treated (exposed to DEET) and untreated (control) gravid females. The rate of egg hatch was considerably reduced after three weeks of retention; this reduction declined to zero for treated and control females at six and four weeks post-treatment, respectively. The fecundity and fertility of gravid female *Ae. albopictus* were affected by the time duration of forced egg-retention. *Journal of Vector Ecology* 30 (1): 45-48. 2005.

Keyword Index: *Aedes albopictus*, oviposition deterrent, *N,N*-diethyl-3-methylbenzamide (DEET), adult survival, fecundity, reproduction, mosquito behavior, mosquito control.

INTRODUCTION

The ability of female mosquitoes to retain some mature eggs after oviposition has been reported by investigators conducting age-determination studies in field populations (Hitchcock 1968, Magnarelli 1975). The egg-retention and oviposition behavior could be influenced by some physical and chemical factors (Dhileepan 1997), including visual, olfactory, and tactile responses (Bentley and Day 1989). In the case of oviposition behavior interrupted by oviposition repellents, or the immediate lack of a suitable aquatic site (medium) for egg laying, the gravid female would be forced to retain mature eggs. It is known that forced egg-retention can affect vitellogenesis (Else and Judson 1972) and oviposition patterns (Chadee 1997) in *Aedes aegypti* (L.), but the effect of duration of egg-retention time on adult survival and reproduction is presently not known.

Bar-Zeev and Ben-Tamar (1968) reported that the insect repellent DEET (*N,N*-diethyl-3-methylbenzamide) provided repellency to ovipositing *Ae. aegypti* for about two weeks. Recently, it was confirmed that DEET at 0.1% rate of application could deter oviposition of *Aedes albopictus* Skuse in containers for two weeks (Xue et al. 2001a). On this basis, DEET was selected as an oviposition deterrent in the present laboratory study to force gravid female *Ae. albopictus* to retain eggs. The relationships of duration of egg-retention time in gravid *Ae. albopictus* with adult survival, fecundity, and egg hatching rates were elucidated. Such basic

information is useful in understanding the survival and reproduction of gravid female mosquitoes and mosquito population dynamics as affected by oviposition deterrents.

MATERIALS AND METHODS

Mosquitoes

The mosquito source for this study was an *Ae. albopictus* strain established in 1992 at the United States Department of Agriculture, Center for Medical, Agricultural, and Veterinary Entomology in Gainesville, Florida, and maintained in the insectary at 27 °C, 70% relative humidity, and 14:10 h L:D photoperiod. Larvae were reared in batches of 200 per plastic tray (30 x 19 cm and 5 cm high and containing 1000 ml well water), and were fed as described in Xue et al. (1995). Adults were provided 10% sucrose on saturated cotton balls in cups. Females were blood-fed on a restrained five- to seven-week-old chicken four to five days after emergence.

Treatments

Six-hundred freshly engorged females were collected from a stock cage and 300 of these females were released into each of two separate cages (each 46 x 38 and 37 cm high). One cage served as treatment and the other as control. The females in each cage had continuous access to 10% sucrose and were held for four days after blood feeding in the insectary conditions described above. Thereafter, two black plastic oviposition cups (each 500 ml capacity), one containing

99 ml of well water plus one ml acetone (control cage) and the other containing 99 ml of well water plus one ml 10% DEET in acetone solution (treatment cage), were provided for oviposition. As a surface for oviposition, a section of white filter paper (24 x 8 cm) was placed against the inside wall of each cup with one end of the paper touching the water to moisten it by capillary action, and the cup placed in the center of each cage. After 24 h, the cups were removed from each cage and the filter papers recovered and dried at room temperature. Eggs deposited on each filter paper were counted; those laid directly in the cup (liquid medium) were also counted and included in the total egg count for the respective cup. The cotton ball with 10% sucrose in each cage was also examined for any egg deposition. After collecting the oviposition cups from each cage, females from the treated cage were collected with the mouth aspirator and released (in groups of 25 each) into ten separate cages. The same procedure was repeated for the control cage; thus, a total of 20 cages (ten treatment and ten control) were established. Sucrose-solution (10%) on cotton ball was provided in each cage and maintained throughout the experiment. Thereafter, at one-week intervals, over a ten-week period, one treatment and one control cage was provided with an oviposition cup for 24 h, after which the number of eggs (on the filter paper and in solution) in each cup was counted. The cotton ball in each cage was also examined as above. Once used in this manner, the cages were excluded from the experiment. Thus, all ten treatment and ten control cages were used in succession over a ten-week period. The experiment was repeated on four different occasions.

Adult survivorship

Adult survivorship was determined by removing and counting dead females from each treatment and control cage at weekly interval (as above) for ten weeks.

Fecundity

The total number of eggs laid on the filter paper and directly in the liquid medium in each oviposition cup in the treatment and control cages (each with 300 females), was counted and recorded at 24 h post-treatment. Thereafter, the ten treatment and ten control cages, each containing 25 females per cage, were monitored weekly in succession over a period of ten weeks post-treatment, as described above. Mean numbers of eggs per female, per cage, was determined by dividing the total number of eggs per cage by the number of surviving females in the cage. The untanned eggs on the filter paper and in solution in each oviposition cup were counted and recorded in accordance with the preceding to assess fecundity.

Fertility

To determine fertility, filter papers from treatment and control cages which had been examined for fecundity were kept moist for three days and then placed in rearing pans containing 500 ml of well water and 50 mg larval food to induce egg hatch. After 48 h, the number of larvae in the pans was observed and recorded to determine percent egg hatch.

In addition, to check for fertility, 30 gravid females that had retained eggs for six weeks were dissected and the spermathecae examined for sperm. Approximately 100 unhatched eggs from these females were also dissected for evidence of any embryonic development.

Data analysis

Percent survival responses were analyzed separately (by time post-treatment) using PROC TTEST ($\alpha = 0.05$; two-tailed test) (SAS 1988). We fit the linear function $y = mx + b$ (y = percent survival, x = time post-treatment, b = y intercept, m = slope) to responses for mosquito survival, natality, and the number of laid eggs that failed to tan, for the treatment and control groups, using PROC REG (SAS 1988), and tested the resulting slope coefficients for homogeneity (Steel and Torrie 1980). For the analysis of natality and laid eggs that failed to tan responses, we omitted all records for which the corresponding fecundity response was null.

To analyze fecundity, we compared responses from the time post-treatment in the control group with the maximum mean number of eggs laid per female (24 h), with the mean number of eggs laid per female, at each time interval post-treatment, in the treatment group using PROC GLM. Dunnett's test (DUNNETTL) was used to determine if any treatment mean was significantly smaller than the control (SAS 1988).

RESULTS

Survival

Mean survival was significantly higher in female *Ae. albopictus* exposed to 0.1% DEET oviposition repellent, compared with females not exposed to repellent, at all times except for weeks three, four, and ten post-treatment, when there was no significant difference (Table 1). Fitted linear model coefficients (\pm SE) for mean percent survival in the treatment group were $m: -0.0404 (\pm 0.0041)$, $b: 110.1657 (\pm 3.9878)$ ($F_{1,43} = 96.46$, $P < 0.001$, $R^2 = 0.70$) and in the control group, $m: -0.0523 (\pm 0.0034)$, $b: 108.5830 (\pm 3.3709)$ ($F_{1,43} = 237.71$, $P < 0.0001$, $R^2 = 0.85$). Slopes for both groups varied by approximately 1.19% and were not significantly different ($t = 0.0994$, $df = 84$).

Fecundity

Female *Ae. albopictus* exposure to oviposition repellents resulted in attenuation of oviposition responses during the ten-week post-treatment period (Table 2). The mean numbers of eggs laid per treatment female were significantly lower at all times post-treatment when compared with control (24 h) females ($F_{11,46} = 45.41$, $P < 0.0001$).

Natality

Overall mean natality responses for treatment ($41.9 \pm 7.3\%$) and control ($71.4 \pm 13.4\%$) females (Table 2) were significantly different ($F_{1,38} = 15.38$, $P = 0.0004$). Fitted linear model coefficients (\pm SE) for mean percent natality in the treatment group were $m: -0.0870 (\pm 0.0058)$, $b: 89.7193 (\pm$

3.5376) ($F_{1,21} = 226.76, P < 0.001, R^2 = 0.92$) and in the control group, $m: -0.0737 (\pm 0.0152), b: 92.9021 (\pm 5.5116)$ ($F_{1,16} = 23.50, P < 0.0002, R^2 = 0.61$). Slopes for both groups varied by approximately 1.33% and were not significantly different ($t = 0.1821, df = 33$).

Number of eggs failing to tan

There was no significant difference in the mean number of eggs failing to tan per female for the treatment (1.13 ± 1.35) and control (0.67 ± 0.91) groups and no significant relationship was found between this value and increasing time for females in the control group (Table 3). However, there was a significant relationship between the numbers of untanned eggs and increasing time for females in the treatment group ($F_{1,21} = 7.62, P = 0.0121, R^2 = 0.28$). Fitted linear model coefficients (\pm SE) in the latter case were $m: 0.0029 (\pm 0.0010), b: -0.4942 (\pm 0.6575)$.

DISCUSSION

Detinova (1962) reported that mosquito ovarioles retaining mature eggs were normal in appearance, but ovarioles with relic eggs changed morphologically in females that had obtained an additional blood meal. The follicle lying above the relic egg changed shape and then showed signs of degeneration. Detinova (1962) suggested that mature eggs retained in ovarioles of mosquitoes might degenerate. This may be one explanation for the reduction in fecundity and the rate of egg hatch after extended egg-retention times that were observed in our study.

The chorion of mosquito eggs at the time of oviposition is soft and white but darkens within four h. If the chorion is not fully tanned within six to eight h of oviposition, the eggs generally collapse when exposed to dry conditions (Clements 1992). Li and Christensen (1993) reported that L-tyrosine, and L-DOPA (L-3, 4-dihydroxyphenylalanine, a phenol oxidase) were involved in the tanning of *Ae. aegypti* eggs and were accumulated in the ovaries during egg development. Our

Table 1. Effects of egg-retention time on mean percent survival following exposure of *Aedes albopictus* to oviposition repellent (0.1% DEET) in the laboratory.

Time post-treatment	Mean percent survival \pm SE ^a	
	Treatment	Control
24 hours	97 \pm 2 a	100 \pm 0 b
1 week	97 \pm 2 a	88 \pm 3 b
2 weeks	96 \pm 3 a	84 \pm 4 b
3 weeks	89 \pm 7 a	84 \pm 2 a
4 weeks	87 \pm 6 a	80 \pm 4 a
5 weeks	87 \pm 3 a	76 \pm 2 b
6 weeks	86 \pm 5 a	72 \pm 5 b
7 weeks	76 \pm 3 a	56 \pm 3 b
8 weeks	64 \pm 10 a	48 \pm 2 b
9 weeks	40 \pm 9 a	16 \pm 6 b
10 weeks	9 \pm 10 a	8 \pm 8 a

^aRow means followed by same letter are not significantly different ($df = 6, \alpha = 0.05$) using PROC TTEST (SAS 1988).

results showed that the number of untanned eggs increased with increased egg-retention time in gravid female *Ae. albopictus*. This may be caused by the retention of mature eggs in the ovaries, preventing the involvement and accumulation of L-tyrosine and other substances related to the tanning of egg chorion.

The rate of egg hatch decreased with increased egg-retention time in gravid female *Ae. albopictus*. Except for the increased number of untanned eggs, we have no explanation as to why the remaining eggs would not hatch after ≥ 6 weeks of retention. Dissection of gravid females that had retained eggs for ≥ 6 weeks revealed that 90% of the retained eggs had no sign of embryogenesis, most likely because of the lack of fertilization; only 33% of the dissected females contained viable sperm in the spermathecae. This may be one reason that eggs did not hatch after retention for ≥ 6 weeks.

Table 2. Effects of egg-retention-time on mean fecundity (number of eggs laid per female) and mean natality (number of eggs laid per female that hatched) following exposure of female *Aedes albopictus* to oviposition repellent (0.1% DEET) in the laboratory.

Time post-treatment	Mean fecundity \pm SE		Mean natality \pm SE	
	Treatment ^a	Control	Treatment	Control
24 hours	3 \pm 2*	73.0 \pm 2.1	0 \pm 0	86.8 \pm 2.5
1 week	51 \pm 12*	3.8 \pm 0.8	70.5 \pm 5.0	83.3 \pm 4.8
2 weeks	52 \pm 8*	3.0 \pm 1.6	63 \pm 2.5	70.0 \pm 16.3
3 weeks	42 \pm 7*	0.7 \pm 0.6	54.8 \pm 5.1	46.8 \pm 29.1
4 weeks	25 \pm 6*	0.7 \pm 1.0	27.0 \pm 4.1	16.5 \pm 20.4
5 weeks	24 \pm 11*	0.1 \pm 0.2	11.3 \pm 4.8	0 \pm 0
6 weeks	14 \pm 3*	0 \pm 0	3.8 \pm 4.8	0 \pm 0
7 weeks	13 \pm 3*	0 \pm 0	0 \pm 0	0 \pm 0
8 weeks	7 \pm 1*	0 \pm 0	0 \pm 0	0 \pm 0
9 weeks	2 \pm 1*	0 \pm 0	0 \pm 0	0 \pm 0
10 weeks	0.4 \pm 0.7*	8 \pm 8	0 \pm 0	0 \pm 0

^aTreatment means for fecundity that are significantly lower than the control mean for 24 h are indicated by * (Dunnnett's test, $\alpha = 0.05$).

Table 3. Effects of egg retention on mean percent of eggs failing to tan following exposure of female *Aedes albopictus* to oviposition repellent (0.1% DEET) in the laboratory.

Time post-treatment	Mean percent eggs failing to tan \pm SE	
	Treatment	Control
24 hours	0 \pm 0	0.2 \pm 0.1
1 week	0.6 \pm 0.8	1.2 \pm 0.8
2 week	0.6 \pm 0.4	0.8 \pm 0.8
3 week	0.6 \pm 0.6	0.6 \pm 1.1
4 week	0.8 \pm 0.3	0 \pm 0
5 week	1.4 \pm 0.5	2.3 \pm 3.9
6 week	6.4 \pm 3.5	*
7 week	10.0 \pm 1.9	*
8 week	17.0 \pm 8.9	*
9 week	44.0 \pm 15.4	*
10 week	11.3 \pm 2.1	0 \pm 0

*Not applicable as no eggs were laid during week.

Aedes albopictus is a container-inhabiting mosquito and an important vector of dengue fever viruses. In our recent laboratory and field studies, we documented that DEET and other insect repellents could induce anti-oviposition, as well as larvicidal effects, for several weeks following treatment of water containers with these compounds (Xue et al. 2001a, b). The present study shows that forced egg-retention in *Ae. albopictus* over time, when induced by application of an oviposition deterrent such as DEET, results in reduced fecundity and fertility in mosquitoes. Thus, effective oviposition deterrents could be useful and developed further in the integrated approach to mosquito control programs against container-inhabiting mosquitoes. Application of oviposition deterrents would not only prevent mosquito oviposition in containers but would simultaneously affect fecundity and reproduction potential, subsequently reducing populations of vector mosquitoes and the incidence of transmission of mosquito-borne diseases.

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