Journal of the American Mosquito Control Association, 11(1):128–132, 1995

OVIPOSITION SITE PREFERENCE OF *AEDES ALBOPICTUS* IN THE LABORATORY

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ABSTRACT. Several parameters on the oviposition site preference of *Aedes albopictus* were studied, including color, container type, salinity, and water type. Dark-colored glass jars, especially black, blue, and red ones were preferred over light-colored jars. The black-colored ovitrap with a paper strip performed better than other types of containers. Seasoned tap water had the highest egg count when compared with a saline water series. In addition, water that had previously been used for the culture of *Ae. albopictus* was the most preferred for oviposition. The significance of this study in conjunction with the present *Aedes* mosquito surveillance and monitoring program is discussed.

INTRODUCTION

Aedes albopictus (Skuse) has been an endemic vector of dengue fever in the Old World (East Asia, the Indian subcontinent, Indo-China, the Pacific islands, and Madagascar) since the 19th century (Fontenille and Rodhain 1989, World Health Organization 1989). Unlike its counterpart *Aedes aegypti* (Linn.), which spreads both classical and hemorrhagic dengue fever in the Old World, the role of *Ae. albopictus* is more limited; it serves occasionally only as a vector of classical dengue fever (Cheong 1986).

With advances in transportation as well as worldwide human and resources movements, *Ae. albopictus* has spread to North and South America in the last decade. Since its first occurrence in the United States was detected in Houston, TX in 1985 (Sprenger and Wuithiranyagool 1986, Hawley et al. 1987, Craven et al. 1988), it has spread to 17 states and 122 counties within just a 5-year period (Francy et al. 1990). Its presence in Brazil had also been noted (Hawley 1988).

In comparison with *Ae. aegypti,* biological studies on *Ae. albopictus* have been scattered (Hawley 1988). Ovipositional site preference studies are important in the planning of a vector control program, especially in the surveillance and monitoring phase. Fay and Perry (1965) had previously reported on the oviposition site preferences of *Ae. aegypti,* indicating that a black-colored beaker with brown blotter and water containing 0.1% methyl butyrate was most preferred by the mosquito. Regarding the oviposition site preference of *Ae. albopictus,* there is thus far only one report on its color preference (Yap 1975a). This investigation is an attempt to explore some parameters involved in the oviposition site preference of *Ae. albopictus* under laboratory conditions. All experimental procedures were essentially similar to the previous study on oviposition site preference of *Toxorhynchites splendens* (Wiedemann) (Yap and Foo 1984).

MATERIALS AND METHODS

Colony: *Aedes albopictus* mosquitoes were colonized from field collections using human bare-leg catches from Penang Island, Malaysia, in 1979. This species has since been cultured in the laboratory at the Vector Control Research Unit, University of Science, Malaysia. Adults are kept in a copper-screened cage (40 × 40 × 40 cm) with a plywood base, and are maintained in an insectary at 26 ± 2°C and 65 ± 10% RH. Sucrose solution (10%) and blood meals from mice are provided for the adult mosquitoes. The immature stages are reared in round enamel trays (30 cm diam × 5 cm deep) in seasoned tap water using an adequate amount of finely ground food powder consisting of beef liver, dog bisuit, and yeast at a ratio of 3:1:1. The seasoned tap water used throughout this study was local tap water that had been kept in storage containers in the laboratory for a minimum of 7 days.

Color preference: Drinking glass jars (7.5 cm rim-diam × 15 cm high) painted outside with glossy white, yellow, green, red, blue, or black paint and without paint (control) were used. The wave length for each color was not determined. A paper strip (12.5 × 2.0 cm, cut from Whatman No. 1 chromatography paper), which served as an oviposition substrate, was placed in each glass jar, which was half-filled with seasoned tap water. The different colored glass jars were then placed in a circle in a large copper-screened cage with a plywood base (76 × 76 × 68 cm).

Fifty pairs (50 males and females each) of newly emerged adults of *Ae. albopictus* aged 5 to 8 days were released into the cages and mice were placed in the cage for 8 h daily (1000–1800 h local time) as a blood meal source for the ovipositing females. The oviposition period was set at 48 h after setting up the oviposition substrates. To avoid positional bias inside the cages, the colored glass jars were shifted one position in a clockwise rotation daily. Such rotational shifts

Table 1. Color preference for oviposition site of *Aedes albopictus* in the laboratory.[1]

Color	% of total eggs collected[2] (mean ± SE)
Black	24.4 ± 6.1 a
Red	22.2 ± 2.1 ab
Blue	20.4 ± 7.0 ab
Green	16.0 ± 2.4 bc
Yellow	11.5 ± 3.1 bcd
White	4.8 ± 2.0 cd
Clear glass (unpainted)	0.8 ± 0.2 d

[1] Four replicates consisted of 100 adults aged 5–8 days (50 males and 50 females). Mean total eggs collected per replicate was 1,935.

[2] Values followed by the same letter are not significantly different ($P > 0.05$) based on one-way ANOVA and LSD tests.

of containers applied to the rest of the parameters studied in this study.

Container preference: Nine types of containers were used: black-colored glass jar with a paper strip (black ovitrap), black-colored glass jar without a paper strip, leaf axil, bicycle tire, bamboo stump, tin can, tree hole, coconut shell, and cement block. Detailed descriptions for each of the containers are reported in Yap and Foo (1984). The container types used were as follows: the glass jar was essentially the same as that mentioned previously; the leaf axil was simulated by using a wooden block (7 × 8.5 × 4.0 cm) with a central hole (5 cm diam × 3.5 cm deep) surrounded by green leaves; the bicycle tire section was 6.5 cm long with a 2.5-cm-wide opening; the bamboo section was 5 cm in internal diameter and 6.0 cm long; the cylindrical tin can was 5.5 cm in internal diameter and 9 cm high; the tree stump was 9.0 cm high with a round surface measuring 11.0 cm in diameter; the halved coconut shell had a rim diameter of 12.0 cm and a 6.5-cm depth at center, and the cement block (9 × 7 × 3 cm) had a central depression of 4 × 4 × 1.5 cm. Laid eggs were recovered by filtering them from water with filter paper (Whatman No. 1 chromatography paper). Eggs were then counted on the filter paper. Throughout the experiment, seasoned tap water was added and maintained at a half-filled position for each of the containers.

Water-type preference: The following water types were tested for their attractiveness for oviposition: seasoned tap water, tap water, rainwater, seasoned water with *Ae. albopictus* larvae, seasoned tap water with *Ae. albopictus* eggs, water previously used for the culture of *Ae. albopictus,* and distilled water. The containers used in this study were black-colored glass jars with a strip of filter paper in each as described earlier.

Table 2. Container-type preference for oviposition by *Aedes albopictus* in the laboratory.[1]

Container type	% of total eggs collected/replicate[2] (mean ± SE)
Black ovitrap with paper strip	26.1 ± 3.4 a
Coconut shell	17.5 ± 4.1 ab
Leaf axil	16.2 ± 4.2 bc
Black ovitrap without paper strip	11.4 ± 1.1 bcd
Tire	10.9 ± 3.7 bcd
Bamboo stump	8.4 ± 3.0 cde
Tin can	4.4 ± 1.7 de
Cement block	3.5 ± 2.3 de
Tree hole	1.7 ± 1.7 e

[1] Four replicates consisted of 100 adults aged 5–8 days (50 males and 50 females). Mean total eggs collected per replicate was 1,836.

[2] Values followed by the same letter are not significantly different ($P > 0.05$) based on one-way ANOVA and LSD tests.

Salinity preference and tolerance of eggs: In addition to the above 3 parameters, a saline water series (40, 20, 10, 3, 1, 0.1, and 0 %), prepared from seasoned tap water and NaCl, was compared using black-colored glass jars half-filled with the different solutions, each jar containing a strip of filter paper. The percentage of eggs hatching at each salinity level was recorded.

For the above parameters, every experiment was replicated 4 times using different batches of mosquitoes and oviposition substrates. All data were converted into percentages and subjected to arc-sine transformation and analyzed with a one-way ANOVA test. Means were separated with Fisher's least significant difference (LSD) test using the StatGraphics statistical program (Statistical Graphics Corporation, New York, USA 1989).

RESULTS AND DISCUSSION

The color preference study indicated that jars painted black, red, and blue were significantly ($P < 0.05$) more preferred by *Ae. albopictus* as oviposition sites, with proportional percentages (mean ± SE) of total eggs collected of 24.4 ± 6.1%, 22.2 ± 2.1%, and 20.4 ± 7.0%, respectively (Table 1). This corresponded well with the earlier field study by Yap (1975a) who reported that black and red were the most preferred colors. The only difference from the earlier study was that blue was also shown to be attractive for oviposition in the laboratory. This may be due to different environmental conditions present in the studies. In the earlier study, the experiments

Table 3. Salinity preference and tolerance of *Aedes albopictus* in the laboratory. Black ovitraps with a strip of paper were used.[1]

Salinity in % NaCl (M NaCl)	% of total eggs collected/ replicate (mean ± SE)[2]	% of eggs hatching
Seasoned tap water	47.2 ± 2.1 a	86.5
0.1 (0.017 M)	29.9 ± 6.3 b	53.1
1 (0.17 M)	22.4 ± 6.5 b	6.9
3 (0.50 M)	0.5 ± 0.2 c	0
10 (1.67 M)	0.2 ± 0.1 c	0
20 (3.33 M)	0 c	0
40 (6.67 M)	0 c	0

[1] Four replicates consisted of 100 adults aged 5–8 days (50 males and 50 females). Mean total eggs collected per replicate was 1,994. A minimum of 1,000 eggs was used to study the salinity tolerance of the eggs.

[2] Values followed by the same letter are not significantly different ($P > 0.05$) based on one-way ANOVA and LSD tests.

Table 4. Water-type preference of *Aedes albopictus* in the laboratory. Black ovitraps with a strip of paper were used.[1]

Water type	% of total eggs collected/ replicate (mean ± SE)[2]
Culture water for *Ae. albopictus*	28.2 ± 8.3 a
Tap water	15.4 ± 3.0 b
Seasoned tap water	12.1 ± 1.2 b
Seasoned tap water with *Ae. albopictus* larvae	11.7 ± 2.6 b
Seasoned tap water with *Ae. albopictus* mosquito eggs	11.2 ± 1.5 b
Distilled water	10.7 ± 3.6 b
Rainwater	10.7 ± 1.4 b

[1] Four replicates consisted of 100 adults aged 5–8 days (50 males and 50 females). Mean total eggs collected per replicate was 1,934.

[2] Values followed by the same letter are not significantly different ($P > 0.05$) based on one-way ANOVA and LSD tests.

were conducted in a sylvan environment under the shaded area of trees and shrubs. The natural *Ae. albopictus* had choices of both the natural breeding habitats as well as the colored-glass jars. Although the same type of blue paint was used in both studies, there may have been a difference in the wave length of blue color used. Our results concur well with those of Fay and Perry (1965) on the oviposition preference of *Ae. aegypti.*

In the container type preference study, various types of containers simulating those from natural to artificial containers were used. The range of container types used took into account previous reports of container breeding sites of *Ae. albopictus,* as follows: black ovitrap (Yap 1975a, 1975b; Yap and Thiruvengadam 1979; Lee 1992), leaf axil (Bonnett 1947), tire (Hawley et al. 1987, Craven et al. 1988, Moore et al. 1988), bamboo stump (Jumali et al. 1979), tin can and other artificial containers (Jumali et al. 1979, Sprenger and Whuithiranyagool 1986), tree hole (Jumali et al. 1979, Lee et al. 1984), and coconut shell (Chan 1986). The black ovitrap with a paper strip contained the highest percentage of total eggs collected per replicate (26.1 ± 3.4%), which differed significantly ($P < 0.05$) from the percentage of eggs collected in all other container types except for coconut shell (Table 2). Next in order of preference were coconut shell and leaf axil (which showed no significant difference in egg counts), tire, and black ovitrap without the paper strip. These results indicate the sylvan nature of *Ae. albopictus,* which breeds in both artificial and natural containers (Yap 1975b, Yap and Thiruvengadam 1979).

In an ovitrap survey conducted on Penang Island, Malaysia, Yap (1975b) obtained satisfactory results using glass jars sprayed outside with glossy black paint and a hardboard paddle as an oviposition substrate. From that study, the author reported that *Ae. albopictus* was present in urban, rural, and forested areas.

As expected, there was a significant difference between the presence and the absence of a paper strip in the black ovitrap (Table 2). The black tire sections without ovipositional strips used in this study did not seem to be highly attractive to the *Ae. albopictus,* although used and discarded tires are known to be good larval habitats for this species (Hawley et al. 1987).

Among the different concentrations of salinity tested, seasoned tap water (control) collected significantly more eggs oviposited by *Ae. albopictus,* with a mean of 47.2 ± 2.1% of the total eggs collected and with 86.5% of the eggs hatching (Table 3). For water with 0.1% and 1.0% NaCl, a considerable number of eggs (29.9 ± 6.3 and 22.4 ± 6.5%, respectively) were collected. However, the percentage of eggs hatching at both these salinity levels, especially those at the 1.0% level was significantly reduced. In this study, it is shown that *Ae. albopictus* prefers saline-free water for oviposition. The use of seasoned tap water or rain water thus optimizes egg collection in ovitraps for *Ae. albopictus.* In comparison to *Ae. albopictus, Ae. aegypti* has been shown to prefer water with low salinity (0.1–1%) compared to saline-free water for oviposition; furthermore, low salinity had no adverse effect on egg hatching in *Ae. aegypti* (Wallis 1954a, 1954b).

The water-type preference study revealed that the culture water for *Ae. albopictus* was most preferred by the mosquito for oviposition (Table 4) with significantly ($P < 0.05$) higher egg counts (28.2 ± 8.3% eggs collected/replicate) than other water types. This confirmed the earlier study by Maire and Langis (1985) who reported the same finding in their work on *Aedes communis* (De Geer).

In Malaysia, the surveillance and monitoring program for *Aedes* mosquitoes relies heavily on the use of ovitraps (Yap 1975a, 1975b; Yap and Thiruvengadam 1979; Anonymous 1986). The use of black-colored ovitraps, each with a hardboard as an egg-laying substrate and half-filled with tap water has also recently been recommended by the World Health Organization for the surveillance of both *Ae. aegypti* and *Ae. albopictus* (World Health Organization 1994). The results from the present study further confirm that a black ovitrap with substratum can be an effective instrument in the surveillance and monitoring program for one of the *Aedes* vectors, namely the predominant and ubiquitous *Ae. albopictus*, especially in the Southeast Asia region.

ACKNOWLEDGMENTS

We would like to thank the following: the Dean, School of Biological Sciences and the Coordinator, Vector Control Research Unit, Universiti Sains Malaysia, Penang, Malaysia, for the use of laboratory facilities; the Malaysian Government R&D program (IRPA), for research support; and the staff of the Vector Control Research Unit, Universiti Sains Malaysia, for technical and administrative support.

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