

EFFECTS OF COLOR AND PERFORATED LID ON OVI TRAP PREFERENCE OF *AEDES AEGYPTI* AND *AEDES ALBOPICTUS*

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ABSTRACT. The shape and color of an ovitrap affect the sampling efficiency of mosquitoes. We examined the effect of perforated lids of different colored cups on the preference of *Aedes aegypti* and *Ae. albopictus* for oviposition containers. The mosquito species were collected from Tuy Hoa City, Phu Yen Province, Vietnam in April 2016. Females of both species laid eggs in cups without a lid in the order of black, red, and green. However, the number of eggs laid by both species was not different between the black cups with a perforated lid and those without a lid. *Aedes aegypti* females laid more eggs in red cups with a perforated lid than cups without a lid. Green cups with a perforated lid were not preferred by *Ae. aegypti* and *Ae. albopictus*. Our results showed that the effect of perforated lids on oviposition preference was dependent on cup color and that it differed between the mosquito species. Our study provides valuable insights for the development of more effective ovitraps for *Aedes* mosquito surveillance and control.

KEY WORDS *Aedes aegypti*, *Aedes albopictus*, color, cover, ovitrap

INTRODUCTION

The yellow fever mosquito, *Aedes aegypti* (L.), and the Asian tiger mosquito, *Ae. albopictus* (Skuse), are important vectors of various arboviruses such as dengue, chikungunya, and Zika viruses (Campbell et al. 2015, Kraemer et al. 2015). Preventing or reducing arbovirus transmission depends entirely on controlling mosquito vectors or interrupting human-vector contact (WHO 2009).

The natural habitats of *Ae. aegypti* and *Ae. albopictus* are tree holes and leaf axils (Christophers 1960, Hawley 1988). Dark, light-absorptive backgrounds of black or brown that are similar to the lining of natural tree-hole cavities might serve as the main attractant for most mosquitoes to select oviposition sites (Torrissi and Hoback 2013). Artificial containers are important breeding habitats for immature *Ae. aegypti* and *Ae. albopictus*. In dengue outbreak areas in Ho Chi Minh City, Vietnam, water containers with inappropriate lids, that is, broken or incomplete covers, increased the risk of infestation by immature *Ae. aegypti* compared with containers with appropriate lids (Tsuzuki et al. 2009). Thus, holes and crevices in a lid, and gaps between a container and a lid, might serve as an entrance for gravid females to oviposition sites.

Ovitraps are a sensitive, low-cost, and valuable tool for epidemiological studies of mosquito-borne diseases (Reiter and Nathan 2001, Paz-Soldan et al. 2016). The shape and color of an ovitrap affect sampling efficiency (Paz-Soldan et al. 2016). Color

preference of *Ae. aegypti* and *Ae. albopictus* for ovitraps has been examined (Yap 1975, Gunathilaka et al. 2018, Dixson et al. 2020). However, little is known about the effect of openings to enter the ovitrap on the color preference of gravid females (Chua et al. 2004). It is necessary to verify the effects of combination of color and openings to develop more effective ovitraps. As there are numerous combinations of shape, size, and position of openings, we focused on a simple, small hole in this study. We used a perforated lid, a lid with a circular hole at the center, to investigate the effect of perforated lids for colored cups on the preference of *Ae. aegypti* and *Ae. albopictus* for oviposition containers.

MATERIALS AND METHODS

Colony establishment and rearing practice

Aedes aegypti and *Ae. albopictus* larvae were collected from Hoa Kiem District, Tuy Hoa City, Phu Yen Province, Vietnam, in April 2016. We collected appropriate 1,000 larvae from households and gardens in suburban areas and transferred them to the National Institute of Hygiene and Epidemiology, in Hanoi, Vietnam. When the adult mosquitoes emerged, *Ae. aegypti* and *Ae. albopictus* adults were separated and introduced into the same species cage to establish the colony.

Two hundred larvae were fed fish food (Hersteller, JBL GmbH, Neuhafen, Germany) in a plastic container (24.0 cm × 17.5 cm × 10.0 cm) containing water (5 cm depth). The adults were moved to a cage (20.5 cm × 20.5 cm × 20.5 cm). Cotton pads soaked in 10% sugar solution were provided as food for the adults. Female mosquitoes were fed blood from mouse for oviposition. The mosquitoes were reared, and all experiments were conducted in a chamber maintained at 27°C, approximately 70% RH, and a 14-h light:10-h dark photoperiod.

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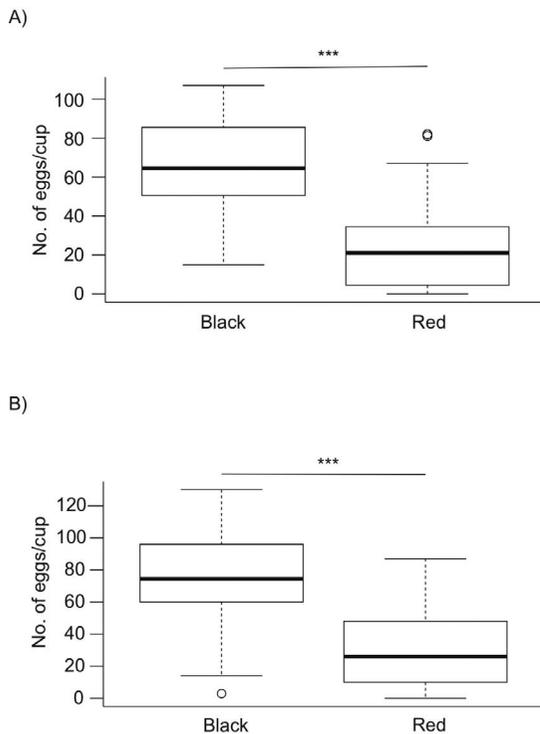


Fig. 1. Boxplot of the number of eggs laid in black and red cups. (A) *Aedes aegypti* and (B) *Ae. albopictus*. Horizontal bar in the middle of the box indicates the median value. Top and bottom of the box indicate the 75th and 25th quartiles. Whiskers denote the maximum and minimum values without outliers. The circles indicate outliers. *** $P < 0.001$.

Two-choice test

The preliminary experiments showed that *Ae. albopictus* laid eggs later and for a longer duration than *Ae. aegypti*. Therefore, we used *Ae. albopictus* 4 days after feeding and *Ae. aegypti* 3 days after feeding. One gravid female was maintained per cage (20.5 cm \times 20.5 cm \times 20.5 cm) (Roberts and Bartholomew 1977). Each cage contained 2 polyvinyl cups (top: 11.5 cm diam, bottom: 9.5 cm diam, height: 8.5 cm) painted with lacquer-type resin coating (black, red, and green). Each cup contained 300 ml of water and 2 sheets of filter paper (17.5 cm width, 4.5 cm height, and folded at each end to fit into the cup) for egg oviposition. The water depth in each cup was 3.5 cm. As females laid eggs a few centimeters above the water surface, we placed the filter paper in the cup so that the bottom edge of the paper was submerged in water. We counted the eggs of *Ae. albopictus* for 4 days and those of *Ae. aegypti* for 3 days. The filter papers were changed every day.

In Experiment 1, we investigated the colored cups in which gravid females laid more eggs. Different colored cups (black and red, red and green, and green and black) were placed in a cage for 1 female to lay

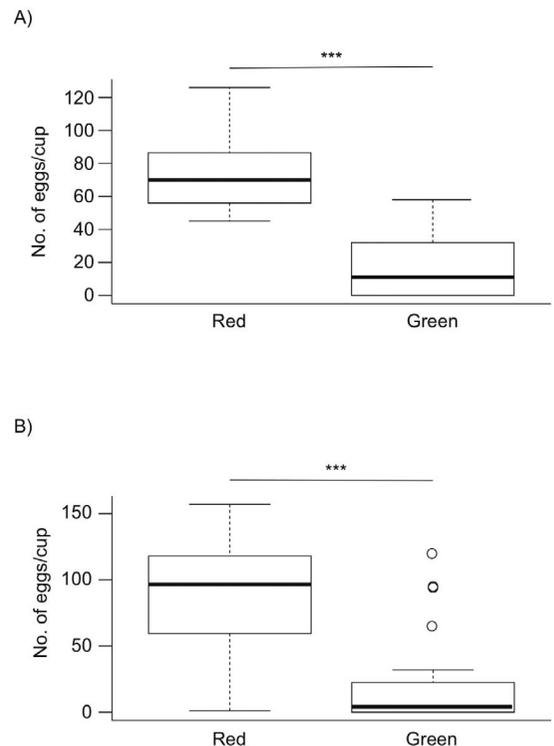


Fig. 2. Boxplot of the number of eggs laid in red and green cups. (A) *Aedes aegypti* and (B) *Ae. albopictus*. Horizontal bar in the middle of the box indicates the median value. Top and bottom of the box indicate the 75th and 25th quartiles. Whiskers denote the maximum and minimum values without outliers. The circles indicate outliers. *** $P < 0.001$.

eggs. At least 30 females were used for each color combination. In Experiment 2, we examined whether the gravid females laid more eggs in cups with perforated lids or cups without lids. The lids were painted the same color as cups (black, red, and green) and had a circular hole (2.5 cm diam) at the center. The lid was placed on top of the cup. The same colored cups, with and without a perforated lid, were placed in a cage for 1 female to oviposit. At least 30 females were used for each color.

Statistical analysis

Although there were numerous eggs, the distribution of some of them was not normal. A Wilcoxon–Pratt signed rank test was performed for the number of eggs laid by gravid females in cups to examine the effect of color and perforated lids. A Kruskal–Wallis rank sum test was used to determine the significant difference in the total number of eggs laid by gravid females to clarify the successful achievement of reproductive potential. In cases where the total number of eggs was significantly different between colors, a pairwise Wilcoxon test was performed for multiple comparisons between cups after the adjustment of P values with

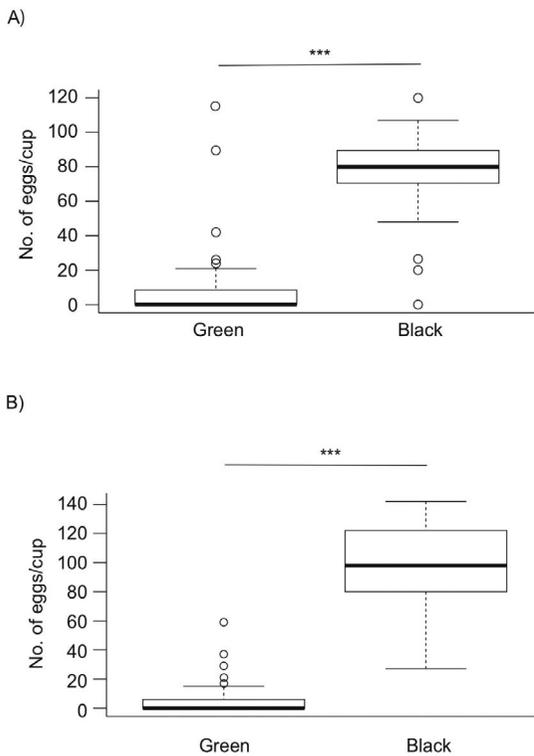


Fig. 3. Boxplot of the number of eggs laid in green and black cups. (A) *Aedes aegypti* and (B) *Ae. albopictus*. Horizontal bar in the middle of the box indicates the median value. Top and bottom of the box indicate the 75th and 25th quartiles. Whiskers denote the maximum and minimum values without outliers. The circles indicate outliers. *** $P < 0.001$.

Bonferroni. All analyses were conducted using R package (R Core Team 2016).

RESULTS

Experiment 1

Females laid more eggs in black color cups than red ($Z = 3.73$, $P < 0.001$ in *Ae. aegypti*; $Z = 3.75$, $P < 0.001$ in *Ae. albopictus*) (Fig. 1A, 1B) and green cups ($Z = 4.78$, $P < 0.001$ in *Ae. aegypti*; $Z = 3.89$, $P < 0.001$ in *Ae. albopictus*) (Fig. 2A, 2B). *Aedes aegypti* and *Ae. albopictus* laid more eggs in red cups than in green cups ($Z = 4.14$, $P < 0.001$; and $Z = 5.01$, $P < 0.001$) (Fig. 3A, 3B). There was no significant difference between the total number of eggs laid by *Ae. aegypti* ($\chi^2 = 0.33$, d.f. = 2, $P > 0.05$) and *Ae. albopictus* ($\chi^2 = 0.02$, d.f. = 2, $P > 0.05$) in different treatments.

Experiment 2

A black perforated lid had no effect on oviposition preference of the mosquitoes ($Z = 0.51$, $P > 0.05$ in *Ae. aegypti*; $Z = 1.86$, $P > 0.05$ in *Ae. albopictus*)

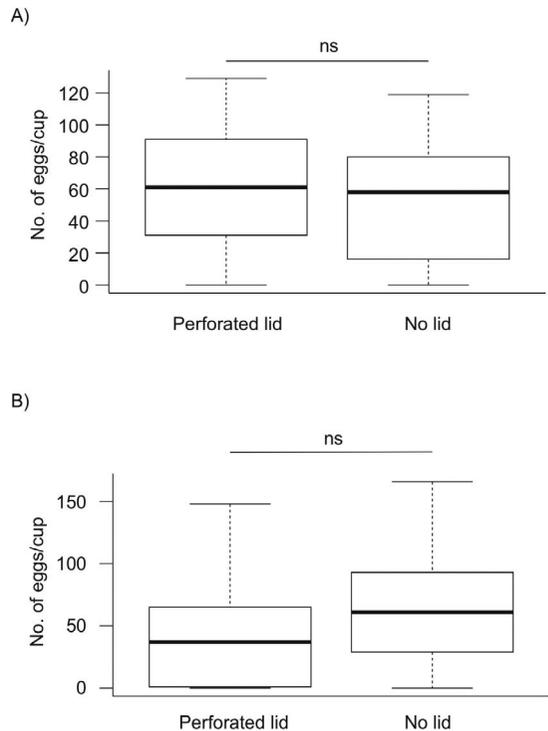


Fig. 4. Boxplot of the number of eggs laid in black cup with a perforated lid and without a lid. (A) *Aedes aegypti* and (B) *Ae. albopictus*. Horizontal bar in the middle of the box indicates the median value. Top and bottom of the box indicates the 75th and 25th quartiles. Whiskers denote the maximum and minimum values without outliers. ns: no significant difference.

(Fig. 4A, 4B). Significantly more eggs were laid by *Ae. aegypti* in red cups with a perforated lid than in cups without a lid ($Z = 4.15$, $P < 0.001$) (Fig. 5A). There was no significant difference in the preference of *Ae. albopictus* between red cups with and without a perforated lid ($Z = 1.37$, $P > 0.05$) (Fig. 5B). Both species laid more eggs in green cups with no lid than in green cups with a perforated lid ($Z = 4.84$, $P < 0.001$ in *Ae. aegypti*; $Z = 4.35$, $P < 0.001$ in *Ae. albopictus*) (Fig. 6A, 6B). In Experiment 2, the results of the Kruskal–Wallis rank-sum test showed the total number of eggs laid by *Ae. aegypti* ($\chi^2 = 24.51$, d.f. = 2, $P < 0.001$) and *Ae. albopictus* ($\chi^2 = 8.91$, d.f. = 2, $P < 0.05$) was significantly different among the different colors. Pairwise comparisons using the Wilcoxon rank sum test showed that the total number of eggs laid by *Ae. aegypti* in green cups was less than that in black ($P < 0.01$) and red cups ($P < 0.001$). The total number of eggs laid by *Ae. albopictus* in green cups was less than that in black cups ($P < 0.05$).

DISCUSSION

In our study, the number of eggs laid by females of both *Ae. aegypti* and *Ae. albopictus* increased in the

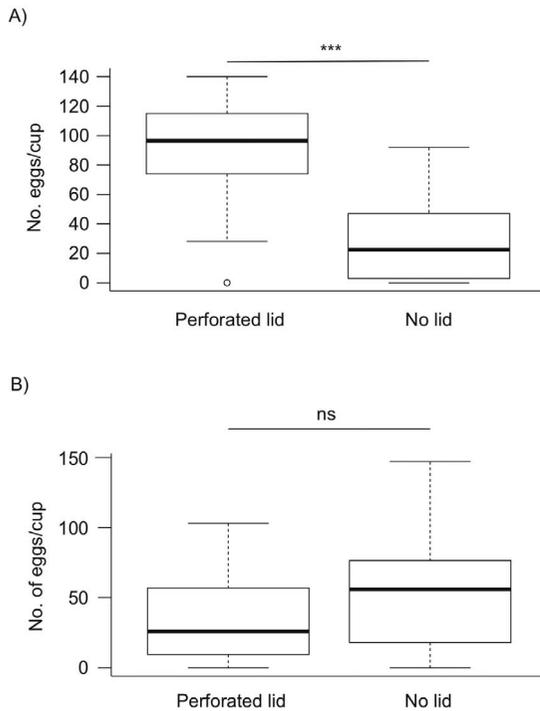


Fig. 5. Boxplot of the number of eggs laid in red cup with a perforated lid and without a lid. (A) *Aedes aegypti* and (B) *Ae. albopictus*. Horizontal bar in the middle of the box indicates the median value. Top and bottom of the box indicate the 75th and 25th quartiles. Whiskers denote the maximum and minimum values without outliers. The circles indicate outliers. *** $P < 0.001$. ns: no significant difference.

following order: black, red, and green. The effect of perforated lids was dependent on the color. Black cups with a perforated lid had no effect on the number of eggs laid in both species. Only red cups with a perforated lid had more eggs than those without a lid in *Ae. aegypti*.

Although green was the least preferred color in our study, significantly more eggs were laid in green cups without a lid than those with a perforated lid. The total number of eggs in green cups with a perforated lid and without a lid (Fig. 6A, 6B) was significantly lower than that in other color cups. Therefore, our results suggest that females of both species might not prefer green cups with a perforated lid.

The order of color preferred by ovipositing females in our study was similar to that reported previously in *Ae. aegypti* and *Ae. albopictus* (Yap et al. 1995, Pavlovich and Rockett 2000). However, according to Gunathilaka et al. (2018), the order of preference was black, green, and red in *Ae. aegypti*. This might be caused by differences in the experimental method, because they used 1,000 mosquitoes and repeated the experiment 3 times. We used 1 female in a cage and repeated the experiment at least 30 times.

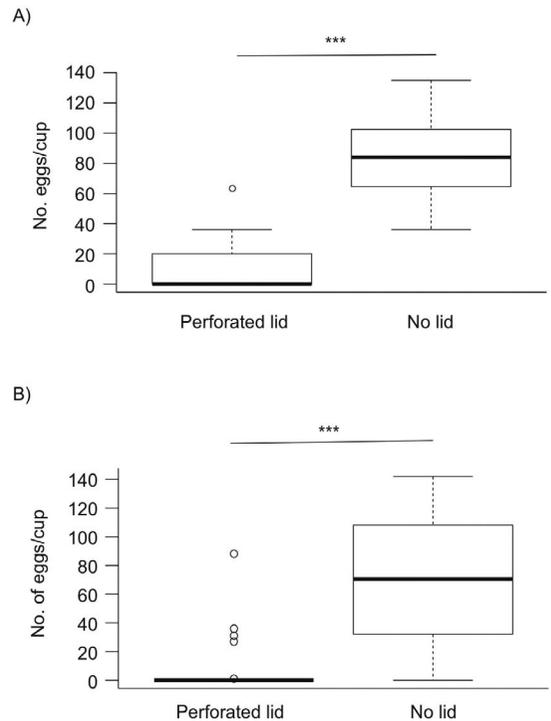


Fig. 6. Boxplot of the number of eggs laid in green cup with a perforated lid and without a lid. (A) *Aedes aegypti* and (B) *Ae. albopictus*. Horizontal bar in the middle of the box indicates the median value. Top and bottom of the box indicate the 75th and 25th quartiles. Whiskers denote the maximum and minimum values without outliers. The circles indicate outliers. *** $P < 0.001$.

Containers with a broken lid caused more infestation of *Ae. aegypti* larvae in Ho Chi Minh City (Tsuzuki et al. 2009). Standard water jars covered with metal lids located outdoors were infested more often with immature *Aedes* mosquitoes than uncovered jars in Thailand (Kittayapong and Strickman 1993), suggesting that *Aedes* females prefer to lay eggs in containers with lids. The infestation ratio might be influenced by the size, shape (such as circle and triangle), position (center or edge and top or lateral), and number (single or multiple) of holes in perforated lids. In our study, the size, shape, position, and number of holes were relatively simple. Further investigation is necessary to determine the effect of these characteristics on oviposition of *Aedes* mosquitoes to develop more effective ovitraps.

Our results indicated that the preference of *Ae. aegypti* for black and green and perforated lids was similar to that of *Ae. albopictus*, although it was different for red. In particular, both species did not prefer green perforated lids. Overall, the efficiency of perforated lid for ovitraps depends on the color and mosquito species. Our study provides insights for the development of effective ovitraps for use in mosquito control and disease surveillance programs.

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