Journal of the American Mosquito Control Association, 13(2):150–152, 1997

RESPONSE OF *CULEX QUINQUEFASCIATUS* TO VISUAL STIMULI

YANG WEN,[1,2] LYNDA E. MUIR[1] AND BRIAN H. KAY[1,3]

ABSTRACT. The vision of *Culex quinquefasciatus* was studied in the laboratory. The attractiveness of colors (black, white, brown, yellow, blue, a skin tone) was evaluated with respect to the collection of *Cx. quinquefasciatus* in natural light, in the dark, and in ultraviolet light. *Culex quinquefasciatus* prefers both black and brown in natural light and ultraviolet light, but, as expected, there is no preference in the dark. There were no significant differences among the other 4 colors in natural light and ultraviolet light.

INTRODUCTION

Worldwide, *Culex quinquefasciatus* Say is a pest when breeding grounds such as sewerage waters are close to human habitation. In China, it is a vector of filariasis and encephalitis, as well as a nuisance mosquito. The principal form of control of *Cx. quinquefasciatus* in Sichuan Province has been the widespread use of chemical insecticides. However, this mosquito has developed resistance to deltamethrin (Kang et al. 1995), and the public has complained that chemical control is ineffective and causes pollution. A new way of controlling *Cx. quinquefasciatus* that is effective and easy to use is being sought.

This study attempted to determine the response of *Cx. quinquefasciatus* to color, so that this information could be used in developing a new trapping method. From previous work, we know that *Anopheles maculipennis* (Meigen) prefers red and *Aedes punctor* (Kirby) and associated species are most attracted by black, red, blue, and brown (Brett 1938). *Coquillettidia perturbans* (Walker) prefers black, red, and blue (Browne and Bennett 1981), and *Ae. aegypti* (Linn.) prefers red and black (Muir et al. 1992a). Das et al. (1993) found that *An. crawfordi* (Reid) and *Ae. lineatopennis* (Ludlow) are attracted by white light, *Cx. bitaeniorhynchus* (Giles) is attracted by blue light, and *Mansonia indiana* (Edwards) is attracted by both. If a successful trapping method can be devised, it could be used for control in populated areas.

MATERIALS AND METHODS

Mosquitoes: A colony of *Cx. quinquefasciatus* was established from larvae collected from a sewerage pond at a water treatment plant. Larvae were fed a mixture of 80% powdered fish food, 10% yeast, and 10% liver powder. Adults were maintained on a diet of fresh apple and 10% sucrose solution. An anesthetized guinea pig was provided for blood meals, and a dish of distilled water was placed in the cage for oviposition. Egg rafts were placed singly into 18 × 12 × 6-cm trays containing 800 ml distilled water. On original collection and periodically thereafter, 3rd- or 4th-instar larvae were identified using the key of Marks (1982). The breeding room photoperiod was set at sunrise at 2300 h and sunset at 1200 h [13:11 h (L:D)], and the temperature and relative humidity were maintained at 27 ± 1°C and 70%, respectively.

Test cage and targets: The test cage (60 × 60 × 60 cm) was made from 6-mm-thick acrylic plastic. An opening (20 × 30 cm) on one side covered by a clear plastic sheet was used to introduce and remove the mosquitoes. Acrylic plastic was used so that CO_2 and other olfactory substances could not enter the cage and affect the experiment, and it allowed maximum ambient light penetration including ultraviolet. All targets (15 × 12 cm) were made of cardboard. Black, white, brown, yellow, blue, and a skin tone were chosen based on the earlier studies by Brown (1954), Browne and Bennett (1981), and Muir et al. (1992a). Brown targets were painted with Chromacryl® (burnt sienna), and skin tone targets were painted with Liquitex® acrylic unbleached titanium (titanium white, raw umber, sienna). The reflectance in the long-wave, ultraviolet, and visible ranges of the spectrum of each target was measured using a Bausch and Lomb Spectronic 20 spectrophotometer. Prior to placement in the test cage, the targets were coated with warmed polyisobutylene adhesive (Five Star Japan Co. Pty. Ltd., Kenmore, Queensland). The positions of the targets on the bottom of the test cage were top left, top right, middle left, middle right, lower left, and lower right.

Experimental design: Each trial consisted of 6 replicates, with the positions of the targets changed for each replicate in a Latin Square design. Mosquitoes 3–10 days old were selected randomly from stock cages. Males and nulliparous females were tested separately. For each replicate, 100–150 mosquitoes were released into the test cage after the targets coated with adhesive were positioned. At the end of the experimental period, the number of mosquitoes stuck on each target was counted.

Trials were conducted under 3 different environmental conditions. In the first trial, under natural light, the test cage was placed in the insectary, which had windows allowing indirect sunlight through (approximately 300–1300 nm, peak around

[1] Queensland Institute of Medical Research, P. O. Royal Brisbane Hospital, Queensland 4029, Australia.
[2] Malaria Unit, Sichuan Institute of Parasitic Diseases, Chengdu 610041, Sichuan, People's Republic of China.
[3] Author to whom correspondence and reprint requests should be addressed.

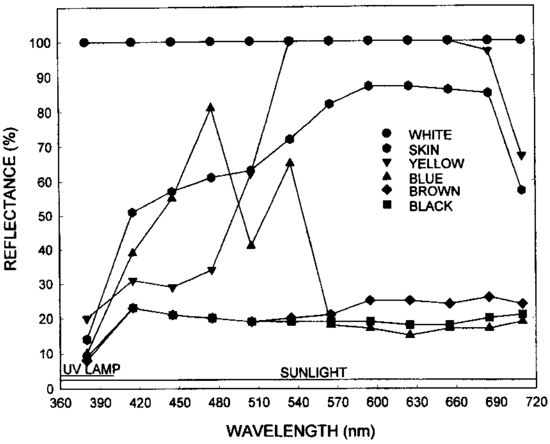

Fig. 1. The spectral reflectances of the 6 colored targets at wavelengths from 380 to 710 nm. The reflectance of white cardboard was taken as 100% and used as the standard to calculate the relative reflectance of the other 5 colors.

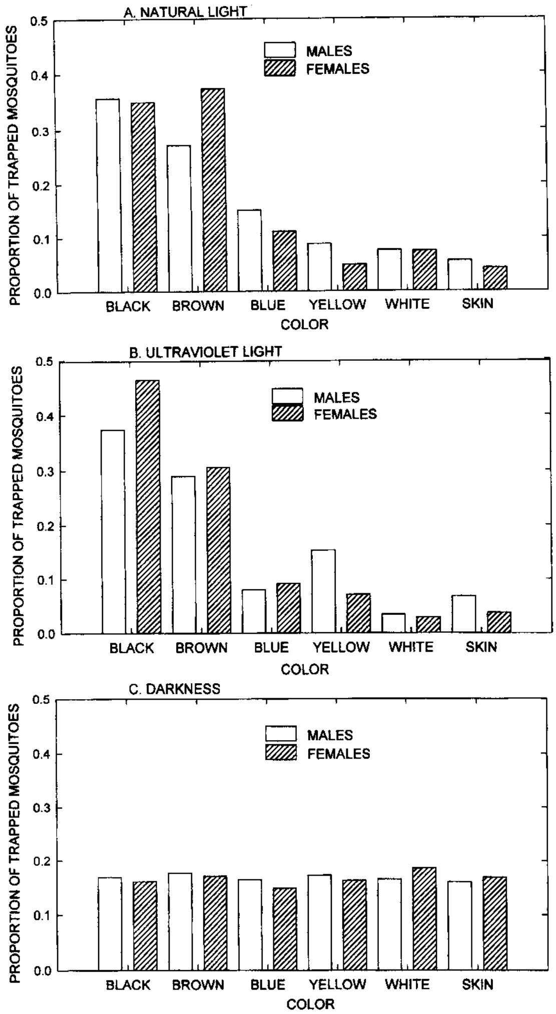

Fig. 2. The proportion of trapped *Culex quinquefasciatus* on each of the 6 colored targets (A) under natural light, (B) under ultraviolet light, and (C) under dark conditions.

500 nm). The temperature and relative humidity were 26°C and 60%, respectively. All artificial lights were turned off when the trial began at 1800 h (sunset), and the trapped mosquitoes were counted at 0900 h (early morning) the following day. The second and third trials (under darkness and ultraviolet light, respectively) were conducted in the breeding room from 1230 h (following sunset) to 2030 h (before sunrise). For the dark trial, the room was not illuminated, and for the ultraviolet light trial, a 15-W ultraviolet light (100% reflective power at 300–400 nm) illuminated the room for the experimental period. The dark trial was undertaken as a control for olfactory and other nonvisual stimuli.

Data analysis: For each trial, a contingency table of the number of mosquitoes trapped on each colored target vs. each position was constructed. Oneway ANOVAs, parametric or nonparametric as appropriate, (SigmaStat Statistical Software, Jandel Scientific) were performed to determine if color or position affected the number of mosquitoes trapped. If an effect was found, the Student-Newman-Keuls method was used for pairwise multiple comparison to determine which color or position was exerting the effect. To investigate whether the sex of the mosquitoes affected the numbers trapped by each colored target, contingency tables of the numbers trapped by each color (with the data from the 6 replicates pooled) vs. sex were constructed and two-way ANOVAs were performed after transforming the data using $\log_{10}(n)$ and $\log_{10}(n + 1)$ as appropriate.

RESULTS

The spectral reflectances of the six colored targets are shown in Fig. 1. The positions of the targets did not affect the number of mosquitoes trapped in any of the trials.

In natural light, the color of the target was a significant factor for the numbers of both male and female mosquitoes trapped (df = 5, H = 27.14, $P < 0.001$; df = 5, F = 45.13, $P < 0.001$, respectively). The orders of attractancy (Fig. 2A) were black > brown > blue > yellow, white, skin tone for males and black, brown > blue, yellow, white, skin tone for females, where > denotes a statistically significant difference ($P < 0.05$).

In the ultraviolet light test also, the color of the target was a significant factor for the numbers of both male and female mosquitoes trapped (df = 5, F = 12.70, $P < 0.001$; df = 5, H = 25.55, $P < 0.001$, respectively). The orders of attractancy (Fig. 2B) were black, brown > blue, yellow, white, skin tone for males and black > brown > blue, yellow, white, skin tone for females.

In the dark-room trial, there was no significant difference between the numbers of mosquitoes trapped on the 6 colored targets ($P > 0.05$) for either males and females (Fig. 2C).

Under both natural and ultraviolet light conditions, there was no significant difference between the numbers of males and females trapped on each color ($P = 0.05$ and $P = 0.07$, respectively) and there were no interactions between color and sex.

DISCUSSION

Our experiments showed that male and female *Cx. quinquefasciatus* preferred to land on black and brown objects compared to blue, yellow, skin tone, and white objects under both natural light and ultraviolet conditions. There was little difference between the numbers of mosquitoes trapped on the black and brown targets because their spectral reflectance patterns were similar. There was no difference in the numbers of *Cx. quinquefasciatus* landing on the blue, yellow, skin tone, and white targets, except for males under natural light conditions, where the number on the blue targets was intermediate between those of the darker and lighter colors. The fact that these colors reflect different amounts of light in different parts of the spectrum raises the question of whether the wavelength or the intensity of light reflected affects mosquito behavior. In the field, areas of low reflectance could indicate shaded or darkened habitat that could afford protection from higher temperatures and lower humidity, usual in direct sunlight.

We have shown previously that mosquitoes see in the ultraviolet (maximum = 345 nm) and green (maximum = 523 nm) ranges of the electromagnetic spectrum (Muir et al. 1992b) and that objects with lower reflectivity in these wavelengths are more attractive to some species of mosquitoes (Brown 1954, Muir et al. 1992a). Our study shows that *Cx. quinquefasciatus* prefers to land on objects of low reflectivity in the wavelength ranges that can be perceived. Under natural light conditions from sunset to after sunrise, the response of the nocturnal *Cx. quinquefasciatus* to different colors was the same as the response of the diurnal *Ae. aegypti* during daylight hours. The proportion of mosquitoes trapped on the different colored targets decreased as the reflectivity of the targets increased. Similarly, under ultraviolet light conditions, the targets reflecting the least light in the ultraviolet range (<400 nm) trapped the most *Cx. quinquefasciatus*.

The polyisobutylene glue used to trap the mosquitoes on the colored targets was effective and long-lasting. More than 90% of the mosquitoes were captured after 16 h in the test cage. Our experiments suggest that low reflective targets coated with this glue will be a cheap and effective way to trap *Cx. quinquefasciatus*, especially if effective olfactory attractants can be developed.

ACKNOWLEDGMENTS

We thank Darryl McGinn (Brisbane City Council Vector Services) and Ian Fanning (Queensland Institute of Medical Research) for help in collecting larvae from the sewerage plant in Brisbane. We also thank Jeff Kibby (Queensland University of Technology) for use of the Spectronic 20 spectrophotometer. This work was supported by the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases.

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