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LABORATORY EVALUATION OF COLORED LIGHT AS AN ATTRACTANT FOR FEMALE *Aedes aegypti*, *Aedes albopictus*, *Anopheles quadrimaculatus*, AND *Culex nigripalpus*

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ABSTRACT

Mosquito feeding activity was monitored in an electronic apparatus (visualometer), having ten ports, illuminated from below with narrow bandwidths of light (700, 650, 600, 550, 500, 450, 400, or 350 nm). Responses of adult female *Aedes albopictus* Skuse, *Ae. aegypti* (L.), *Anopheles quadrimaculatus*, Say and *Culex nigripalpus* Theobald to feeding stations (blood containers) over each light port. No-light and broad spectrum white light were used as controls. Color preferences were based on electronic detection of feeding times. *Aedes aegypti* showed no significant feeding preferences over any of the colors. Conversely, *Ae. albopictus*, *An. quadrimaculatus*, and *Cx. nigripalpus* showed preferences for several of the wavelengths of light. In decreasing order, *Aedes albopictus* fed significantly longer at 600 nm, 500 nm, white, 450 nm, 400 nm, and black. For *An. quadrimaculatus*, significantly longer feeding durations were found over the black or white controls and all other individual wavelengths had significantly longer feeding durations than 350 nm. Finally, in decreasing order, significantly greater feeding times were recorded for *Cx. nigripalpus* over 500 nm, 600 nm, 450 nm, white, 650 nm, and 550 nm compared to the other wavelengths tested.

Key Words: mosquito, attractants, visual ecology, color vision, visual spectrum

RESUMEN

La actividad de la alimentación de mosquitos fue estudiado en un aparato electrónico (medición visual), que tiene diez puertos, iluminados por debajo con secciones estrechas de luz de banda-ancha (700, 650, 600, 550, 500, 450, 400, o 350 nm). La respuesta de hembras adultas de *Aedes albopictus* Skuse, *Ae. aegypti* (L.), *Anopheles quadrimaculatus* Say y *Culex nigripalpus* Theobald a las estaciones de alimentación (recipientes de sangre) sobre cada puerto de luz. Sin luz y luz blanca de ancho espectro fueron usados como controles. Las preferencias de color fueron basadas sobre la detección electrónica de los tiempos de alimentación. *Aedes aegypti* no mostro preferencia significativa de alimentación para ninguno de los colores. Al contrario, *Ae. albopictus*, *An. quadrimaculatus*, and *Cx. nigripalpus* mostraron preferencias para varios de las ondas de luz. En orden decreciente, *Aedes albopictus* se alimento significativamente mas tiempo A 600 nm, 500 nm, blanca, 450 nm, 400 nm, y negra. Para *An. quadrimaculatus*, la duración de tiempo de alimentación fue significativamente mas largo en los controles de negro y blanco y todas las otras ondas tenian una duración de alimentación mas larga que 350 nm. Al fin, en orden decreciente, el tiempo de alimentación fue significativamente mayor para *Cx. nigripalpus* sobre 500 nm, 600 nm, 450 nm, blanca, 650 nm, y 550 nm comparado con las otras ondas probadas.

That some species of mosquitoes and other medically important Diptera are attracted to artificial light or other visual stimuli has long been known and exploited in a variety of trap designs. Not all mosquito species respond equally to visual stimuli or to different wavelengths of light. Indeed, many mosquitoes do not respond to light traps at all (Service 1993). Mating, dispersal, appetitive flight, and location of sugars, hosts, resting, oviposition and overwintering sites all are governed to some degree by vision. Many authors have examined the important visual components

of host/resource finding and have divided them into shape, color (reflected and transmitted), size, contrast, light intensity, texture and movement (Allan et al. 1987). These factors alone or in combination appear to play an important role in a female's ability to successfully locate a suitable host or other resource.

Much of the mosquito research on color attraction has evaluated the response of diurnal species to reflected light colors (Brett 1938; Brown 1954; Granger 1970; Browne & Bennett 1981). Studies using colored transmitted light are few, and even

fewer provide information on individual species or emit light of known wavelengths and/or intensity (Headlee 1937; Breyer 1963; Bargren & Nibley 1956; Gjullin et al. 1973; Wilton & Fay 1972; Vavra et al. 1974; Browne & Bennett 1981). None of these studies incorporates both reflected and transmitted light. Lack of information about the attractiveness of different light wavelengths for different species of mosquito is a serious void in a science where mosquito control/research operations are based largely on the numbers and types of mosquitoes captured in light-baited traps.

A laboratory method for the evaluation of the relationship between various light colors (wavelengths) of transmitted/reflected light and feeding preference (based on duration of feeding time in seconds) is presented herein for lab reared *Aedes albopictus* Skuse, *Ae. aegypti* (L.), *Anopheles quadrimaculatus*, Say (Type A) and wild *Culex nigripalpus* Theobald. Information obtained about mosquito responses to different wavelengths of light can be used to further exploit insects' attraction to artificial light and enhance our ability to conduct studies on population dynamics, species specific surveys and/or improve reduction strategies.

MATERIALS AND METHODS

Visualometer and Data Collection

A pie-shaped olfactometer (Butler & Katz 1987; Marin et al. 1991; Wilson et al. 1991; Butler & Okine 1995; Okine 1994) electronically quantifies insect feeding activity on 10 compounds simultaneously for a set time period (Fig. 1a, b). Ten holes drilled into the bottom of the aluminum pie-shaped arena contained the tips of fiber optic cables that emitted light upwards and illuminated the artificial hosts from below (Fig. 1b, N). The fiber optic tips were covered with recessed interference filters (described below). As an additional attractant, CO₂ (0.5 l/min) was released through Tygon® tubing (Norton Performance Plastics Corp., Akron, OH), positioned directly below each artificial host (Fig. 1, G) for measured time intervals of 4 s "on" and 6 s "off". The visualometer was located in a temperature-controlled, light-proof, Faraday-cage room (Lindgren Enclosures, Model No. 18-3/5-1). The apparatus (hereafter called a "visualometer") was modified to compare 10 different light wavelengths that illuminated from below identical feeding stations. Each feeding station was illuminated with unique wavelengths (ca. 10 nm width) produced using filtered broad spectrum white light. The mosquito feeding time on the illuminated feeding stations was recorded, logged, and analyzed using touch and bite contact seconds created when the mosquito closed a circuit (Fig. 1, K and J).

Feeding Stations

The food source contained within the feeding stations used in the visualometer, consisted of fresh, citrated bovine blood mixed with agar and various feeding stimulants/attractants. The food mixture contained 1.66 g agar (U.S. Biochemical Corp., Cleveland, OH), 33 ml fresh citrated bovine blood; 100 ml deionized water, 7.14 mg sodium chloride, 0.38 mg potassium chloride, 0.154 mg calcium chloride dihydrate, 0.2 mg magnesium chloride hexahydrate, 0.42 mg dibasic sodium phosphate, 2.1 mg sodium bicarbonate, 0.92 mg dextrose, and 0.184 mg glutathione disulfide (oxidized glutathione), and was made to a final volume of 133 ml and adjusted to a final pH of 7.4. The blood/agar/feeding mixture was placed into the "cup" on the underside of a 35-mm plastic film canister lid where it was covered with a reinforced silicone membrane (Butler et al. 1984) held in place using a 4-mm retaining ring cut from the top of the film canister. The feeding station was then inserted into 1 of the 10 holes cut into the transparent plexiglass visualometer lid. Between trials, the visualometer was disassembled and washed. Feeding stations were replaced for each replicate and new mosquitoes were used for each trial.

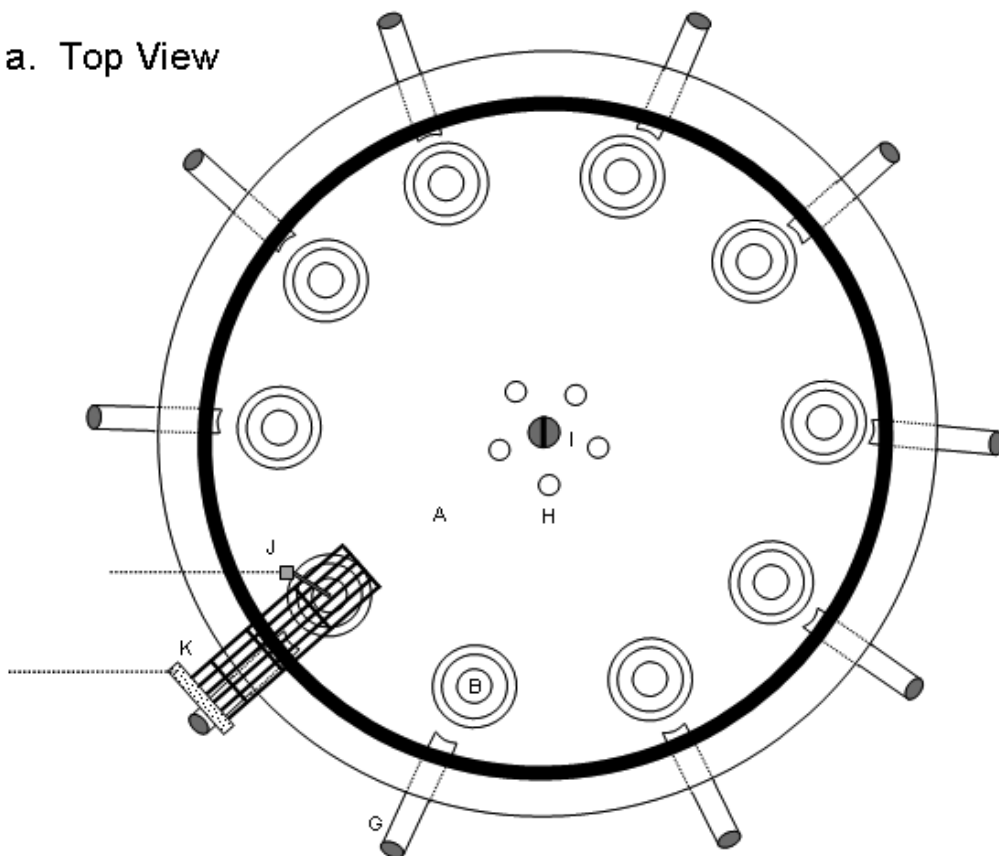
Light Source and Filters

The light source was a wide spectrum tungsten-halogen bulb (Sylvania, no. DNF, Danvers, MA) transmitted through fiber optic cables (RTS Industries, Gainesville, FL) (Fig. 1b, N). Seven VIS-NIR broadband (± 5 nm) interference filters (350, 400, 450, 500, 550, 600, 650, and 700 nm) (Fig. 1b, L) with appropriate neutral density filters (Fig. 1b, M) to equalize intensities were used for each wavelength (Oriol Instruments, Stratford, CT). The "white" light (with neutral density filter) from the fiber optic cable and no light were used as controls.

Mosquito Species

Each trial used 150, 5-to-8d-old nulliparous, non blood-fed females aspirated from cages containing both male and females with sugar (1.0 M) provided *ad libitum*. *Aedes albopictus*, *Ae. aegypti*, *An. quadrimaculatus*, and *Cx. nigripalpus* were the species evaluated. Each species was tested separately. Laboratory colonies maintained at the Center for Medical, Agricultural and Veterinary Entomology (USDA, ARS), in Gainesville, FL provided recently colonized (1995) *Ae. albopictus*, and specimens from a long-established colony of *An. quadrimaculatus* (Type A). *Aedes aegypti* reared as outlined in Gerberg (1970) were obtained from an established University of Florida departmental colony. Wild *Cx. nigripalpus* were reared from larva and pupae obtained from a sewage lagoon at

1a. Top View



1b. Side View

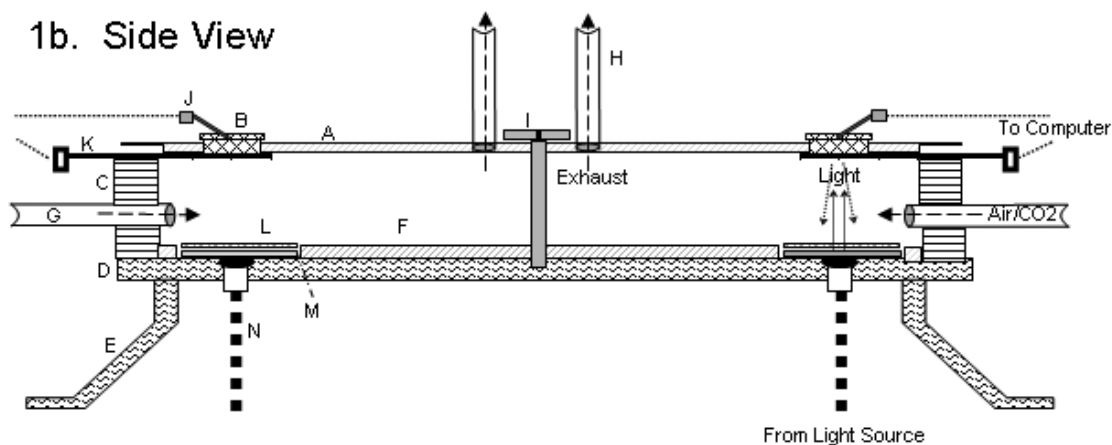


Fig. 1. Visualometer (a) Top view (b) Side view. (A) 430 × 5-cm dia. transparent plexiglass lid (B) Artificial host, (35-mm film canister lid) (C) 30-cm high plastic side piece with holes for tygon tubing (D) Aluminum base with holes for fiber optic cables (E) Support leg (F) Plexiglass filter support (G) 10 cm diameter Tygon® tubing for incoming air/CO₂ (H) Tubing for exhaust (I) Assembly screw (J and K) Probe inserted into top of artificial host and bottom sensor fitted under artificial host (feeding mosquitoes complete circuit logged by computer), one sensor per artificial host (L) Interference (bandpass) filter (M) Neutral density filter (N) Fiber optic cable (attached to light source). Note, drawing not to scale.

the University of Florida Swine Research Unit. All mosquitoes were reared and maintained at 25°C, 95% RH and a 14:10 (L:D)H photoperiod. All trials were run from 1600 to 0800 h.

Statistical Analysis

Touch/bite contact seconds were recorded for 16 h. All species trials were analyzed using the first 4 h of feeding activity, with the exception of *Cx. nigripalpus*, in which the last 4 h of feeding times were analyzed. A 10 × 10 Latin square design (3-way ANOVA) was used for *An. quadrimaculatus*. For other species, a randomized complete block (2-way ANOVA) design with 8 to 10 replications was used. Duncan's multiple range test was used to delineate significant differences between the colored light treatment means. Differences between treatment means were considered significant at $\alpha = 0.05$. Data were log (X+1) transformed prior to analysis.

RESULTS

With the exception of *Cx nigripalpus*, all species showed a period of "orientation/ acclimation" lasting ca. 10-15 min, after which mosquitoes would begin aggressively probing and feeding on the artificial hosts. Of these, *Ae. albopictus* was the least aggressive and consequently had the lowest over all feeding times on the different feeding station/color combinations. The wild *Cx. nigripalpus* presumably still under circadian control did not begin actively feeding until about 4 h into the trial.

Aedes aegypti. Feeding duration (Fig. 2) results for this species were not different for feeding times ($F = 1.48$, $df = 9$, $P = 0.17$) over any of the colors tested. Differences (day effect) for total seconds of feeding ($F = 2.06$, $df = 9$, $P = 0.04$) were observed for different replications.

Aedes albopictus. This species showed preferences ($F = 2.59$, $df = 9$, $P = 0.03$) for certain wavelengths of light (Fig. 2). *Aedes albopictus* fed longer on yellow-orange (600 nm), blue-green (500 nm), white, blue (450 nm), violet (400 nm), and black compared to other colors tested. *Aedes albopictus* had an overall mean (\pm SEM) feeding time of 244 ± 44.2 s which was significantly lower than the feeding times ($F = 9.74$, $P < 0.01$) of the other mosquito species. As with all other trials, significant differences for total feeding durations ($F = 3.27$, $df = 9$, $P < 0.01$) were observed for different replications.

Anopheles quadrimaculatus. Feeding duration (Fig. 2) results for this species showed slight differences for feeding times ($F = 1.74$, $df = 9$, $P = 0.05$) where white and black controls were the greatest. All other individual wavelengths had significantly longer feeding durations than 350 nm. No differences for total seconds of feeding

were observed for different replications ($F = 1.87$, $df = 9$, $P = 0.07$) or positions ($F = 0.67$, $df = 9$, $P > 0.73$).

Culex nigripalpus. Due to lack of activity during the first 4 h of the feeding trials, the last 4 h (2000-2400) were analyzed and presented. Significant color preferences ($F = 1.94$, $df = 9$, $P = 0.04$) were observed for this species (Fig. 2) were in decreasing order, blue-green (500 nm) orange (600 nm), blue (450 nm), white, red (650 nm) and yellow-green (550 nm) were preferred over the other colors tested.

DISCUSSION

Considering the variation in attractiveness of different mosquito species to light-baited traps (Huffaker & Back 1943; Bidlingmayer 1967), it is not unreasonable to expect that individual species will vary in wavelength preference. Such wavelength preferences (exhibited by behavioral responses) may or may not correspond to spectral sensitivities. For attraction to light-baited traps, intensity is considered more important than color (Barr et al. 1963). As such, many studies of color light preferences in Diptera are criticized because they fail to compensate for intensity (and/or hue) and make interpretation of the results difficult (Allan et al. 1987). These visualometer tests compensated for variations in light intensity by incorporating neutral density filters at each wavelength so that each treatment only varied by color and an accurate assessment of "color" preference could be obtained. Even so, different wavelengths may be physiologically more stimulating and result in greater behavioral responses.

For mosquitoes, electroretinograph studies for determining spectral sensitivities have been published for only *Ae. aegypti* (Muir et al. 1992; Snow 1971). These electroretinograph studies provide evidence of bimodal sensitivities showing a small peak at 350 nm and a large peak at 550 nm. This bimodal pattern is similar to those found for tabanids (Smith 1986; Allan et al. 1991) and other insects (White 1985), and is assumed, but never tested, to be similar to the spectral sensitivities of other mosquito species. Interestingly, spectral sensitivity research has focused mainly on diurnal species that are not generally attracted to standard light-baited traps. In our visualometer trials *Cx. nigripalpus* is the only species commonly captured in broad spectrum light baited traps (e.g., CDC style). Results of our trials showed none of the mosquito species tested were highly attracted to both 350 and 550 nm and over the other wavelengths tested. Peak spectral sensitivities of approximately 350 and 550 nm may serve to allow discrimination in an environment dominated by greens and blues (Lythgoe 1979), but do not necessarily correspond to attractive wavelengths.

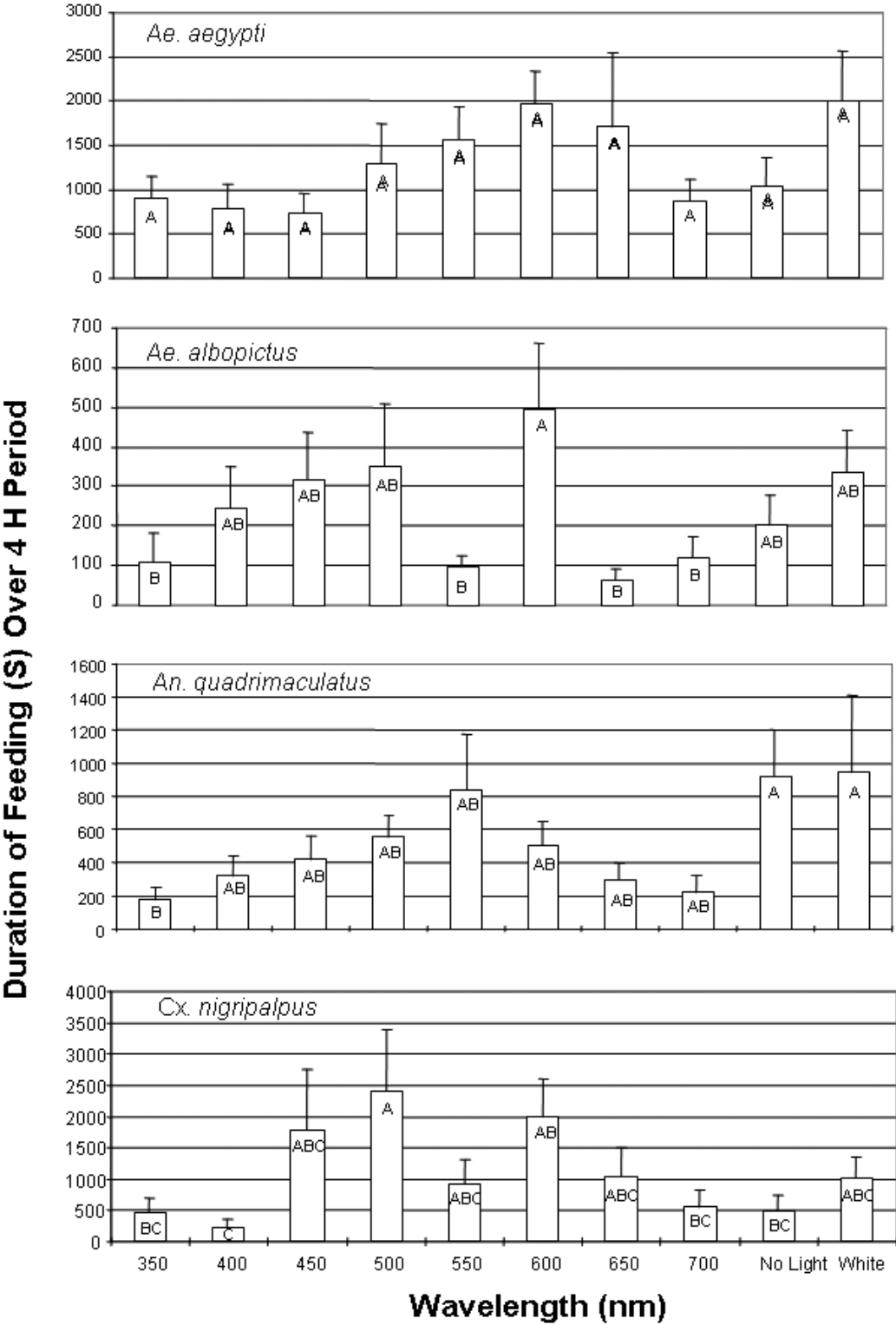


Fig 2. Duration of feeding (seconds) during a 4 h exposure (means \pm SEM) for *Ae. aegypti*; *Ae. albopictus*; *An. quadrimaculatus*, and *Cx. nigripalpus* on artificial hosts illuminated with different wavelengths of light. Means within each species group with the same letter are not significantly different ($\alpha = 0.05$, Duncan's Multiple Range Test).

Aedes aegypti and *Ae. albopictus* are not captured frequently in mosquito traps baited primarily with light (Service 1993). Because these species are diurnal, reflected light appears to be more important in resource location than transmitted light. In general, most successful *Ae. aegypti/albopictus* adult traps do not use light, but rather rely on strategic placement and low reflective colors (Fay 1968; Freier & Francy 1991). The relatively small numbers of *Ae. aegypti/albopictus* captured in light traps indicates that transmitted light is relatively unimportant in host/resource choice. Indeed, the duration of feeding times for *Ae. aegypti* did not differ significantly among wavelengths tested. Duration of feeding times for *Aedes albopictus* were significantly greater for 600 nm, 500 nm, broad spectrum white, 450 nm and 400 nm. Field trials with light emitting diodes or other sources of monochromatic light might result in similar attractive colors under field conditions.

Although nocturnally active, *An. quadrimaculatus* is another species poorly collected by light-baited traps (Bradley 1943). In our visualometer trials, *An. quadrimaculatus* was attracted most strongly by the contrasting "no light" and broad spectrum white controls followed by 550 nm light. Although ultraviolet lamps have long been known to increase the numbers of host or resource seeking mosquitoes captured at light traps (Headlee 1937; Weiss 1943; Williams et al. 1955; Breyev 1963), 350 nm was the least attractive wavelength for *An. quadrimaculatus* and most of the other species tested in our study.

If the duration of feeding is a measure of attractiveness, then the feeding time results for *An. quadrimaculatus* differed slightly with those found in two field experiments using colored light emitting diodes which found no significant trap count differences for *An. quadrimaculatus* (Burkett et al. 1998). In either case, the color of light does not appear to be important in the host/resource seeking behavior of *An. quadrimaculatus* based on these studies.

With the latter being more effective, field trials with CDC-type light traps baited with light and those with light and CO₂ are effective at collecting *Cx. nigripalpus* (Nayar 1982). Field research using narrow wavelength LEDs (Burkett et al. 1998) also found this mosquito attracted to light traps, and in one field trial, *Cx. nigripalpus* was significantly attracted to green (567 nm) followed by blue (450 nm) and white over the other colors tested. This largely agrees with what was found in the visualometer trials for this species where this mosquito was more attracted to the green when compared to the other colors. Given the weakly significant results, and general lack of supporting field data, light color is largely unimportant in host/resource acquisition for both *Cx. nigripalpus* and *An. quadrimaculatus*.

Future trials with the visualometer need to concentrate on species known to be attracted to artificial light. Information obtained about medically important mosquitoes can be used to further improve current light-based trapping methods and, ultimately, enhance studies on their population dynamics, surveys of species, and to improve reduction strategies.

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