USE OF AN ARTIFICIAL BROMELIAD TO SHOW THE IMPORTANCE OF COLOR VALUE IN RESTRICTING COLONIZATION OF BROMELIADS BY *AEDES AEGYPTI* AND *CULEX QUINQUEFASCIATUS*[1]

J. H. FRANK[2]

ABSTRACT. An artificial bromeliad was developed which, painted and containing an infusion water, elicited ovipositional response by caged adult *Aedes aegypti*, *Culex quinquefasciatus*, *Wyeomyia vanduzeei* and *Wy. mitchellii*. Comparison was made of the ovipositional response of adults of the four mosquito species to artificial bromeliads painted black, white, dark green and deep blue. Adult *Ae. aegypti* and *Wy. vanduzeei* did not discriminate significantly between white, dark green and deep blue, but whereas *Ae. aegypti* showed a preference for black, *Wy. vanduzeei* showed an aversion to black. Adult *Wy. mitchelli* responded similarly to *Wy. vanduzeei* except that although deep blue was preferred to black, it elicited a significantly weaker response than did dark green and white. Adult *Cx. quinquefasciatus* responded similarly to *Ae. aegypti* but did not show a significant preference for black over dark green. The high color value (i.e., lightness) of natural bromeliad leaves is likely to deter oviposition by adult *Ae. aegypti* and *Cx. quinquefasciatus* in favor of competing oviposition sites of lower color value.

INTRODUCTION

Aedes aegypti (Linn.) and *Culex quinquefasciatus* Say formed only a small minority of preimaginal stages of mosquitoes in water-containing leaf axils of the imported bromeliad *Billbergia pyramidalis* (Sims) Lindley in four cities in southern Florida.[3] The great majority of the preimaginal mosquitoes were *Wyeomyia vanduzeei* Dyar and Knab and *Wy. mitchellii* (Theobald). Since *Ae. aegypti* and *Cx. quinquefasciatus* are common urban mosquitoes in southern Florida, an explanation was sought for their relative scarcity in *B. pyramidalis* axils. Potential explanations were that *Ae. aegypti* and *Cx. quinquefasciatus* females selected other sites in preference to *B. pyramidalis* axils, the least complex explanation, or that conditions for survival of the preimaginal stages of these mosquitoes were especially poor in *B. pyramidalis* axils. The most obvious difference between the bromeliads and other oviposition sites used by *Ae. aegypti* and *Cx. quinquefasciatus* was color. Thus, experiments to test preference for color of oviposition sites were made using the four species mentioned.

In selection of an oviposition site, females of *Anopheles atroparvus* van Thiel avoided green in favor of red and blue (Bates 1940), and females of *Aedes triseriatus* (Say) avoided yellow in favor of red, brown, green, purple and blue (Williams 1962) and preferred amber to green (McDaniel et al. 1976). Females of *Toxorhynchites splendens* (Wiedemann) preferred red to blue, green and yellow (Yap and Foo 1984). In experiments with *Ae. aegypti* (Beckel 1955, Williams 1962, O'Gower 1963), *Aedes hexodontus* Dyar (Beckel 1955), *Ae. triseriatus* (Williams 1962), *An. atroparvus* (Bates 1940), *Anopheles quadrimaculatus* Say (Lund 1942), *Tx. splendens* (Yap and Foo 1984), and both subspecies of *Tx. rutilus* (Coquillett) (Slaff et al. 1975, Hilburn et al. 1983), females showed a preference for black or dark colors over white or light colors. Preference for dark oviposition sites led to the development of a black oviposition trap for *Ae. aegypti* (Fay and Perry 1965, Fay and Eliason 1966) which also is effective for *Cx. quinquefasciatus* when provided with hay infusion water (Frank and Lynn 1982). However, Istock et al. (1983) obtained oviposition by *Wy. smithii* (Coquillett) in containers fitted with white, green, and purple-veined-green papers simulating leaves of the pitcher plant *Sarracenia purpurea* L.

Color preferences of the bromeliad-inhabiting *Wy. vanduzeei* and *Wy. mitchellii* had not been investigated. Leaves of almost all bromeliads bear peltate trichomes which, at least in many tillandsioid species, absorb nutrients (Benzing 1980). Some bromeliad leaves with elevated trichomes and thus an air layer between the leaf surface and trichome surface, appear silvery white due to reflectance. If trichomes are not elevated, or if air is displaced by water when the leaf surface is wet, then the leaves appear more green and less white. Thus, in general, the water-holding axils will appear greener than the rest of the leaf surfaces where considerable white light may be reflected.

MATERIALS AND METHODS

After unsuccessful attempts with other materials, an artificial bromeliad model was devel-

---

[1] University of Florida, Institute of Food and Agricultural Sciences, Journal Series No. 5656.

[2] Florida Medical Entomology Laboratory, 200 9th Street S.E., Vero Beach, FL 32962.

[3] Frank, J. H. 1983. Mosquitoes in the man-made habitat created by imported bromeliads in Florida. Paper presented at 39th Annu. Mtg. of Am. Mosquito Control Assoc. (Lake Buena Vista, FL).

oped (Fig. 1) which was acceptable in oviposition trials to caged *Wyeomyia* mosquitoes. Such models were made easily and inexpensively and were painted with gloss enamel spray paints. The commercially-available spray paints used in the following trials were characterized in the three color dimensions [value, chroma, hue (Fig. 2)] by the name adopted by the U.S. National Bureau of Standards (Kelly and Judd 1955) and by Munsell alphanumerical designation (Munsell 1966) as black (N 1/), white (N 9/), deep blue (5 PB 3/10) and dark green (5G 3/6). Manufacturers' product identifications were: black (KRYLON 1601 glossy black), white (KRYLON 1501 glossy white), deep blue (RUST-OLEUM 7727 royal blue) and dark green (KMART U3734 green). On the scale of value (darkness-lightness) black was at step 1, blue and green at step 3, and white at step 9. On the scale of chroma (intensity) black and white were at step 0 (neutral), green was at step 6, and blue at step 10. Two hues were used: green and blue. After painting, the models were allowed to dry for several weeks until the odor of paint was no longer detectable to the investigator.

Wyeomyia vanduzeei and *Wy. mitchellii* adults used were from a laboratory colony originating from Vero Beach and with occasional addition of wild-caught larvae and pupae. *Aedes aegypti* adults were collected as larvae in a tire dealer's yard at Vero Beach in November 1983. *Culex quinquefasciatus* were collected as eggs in an

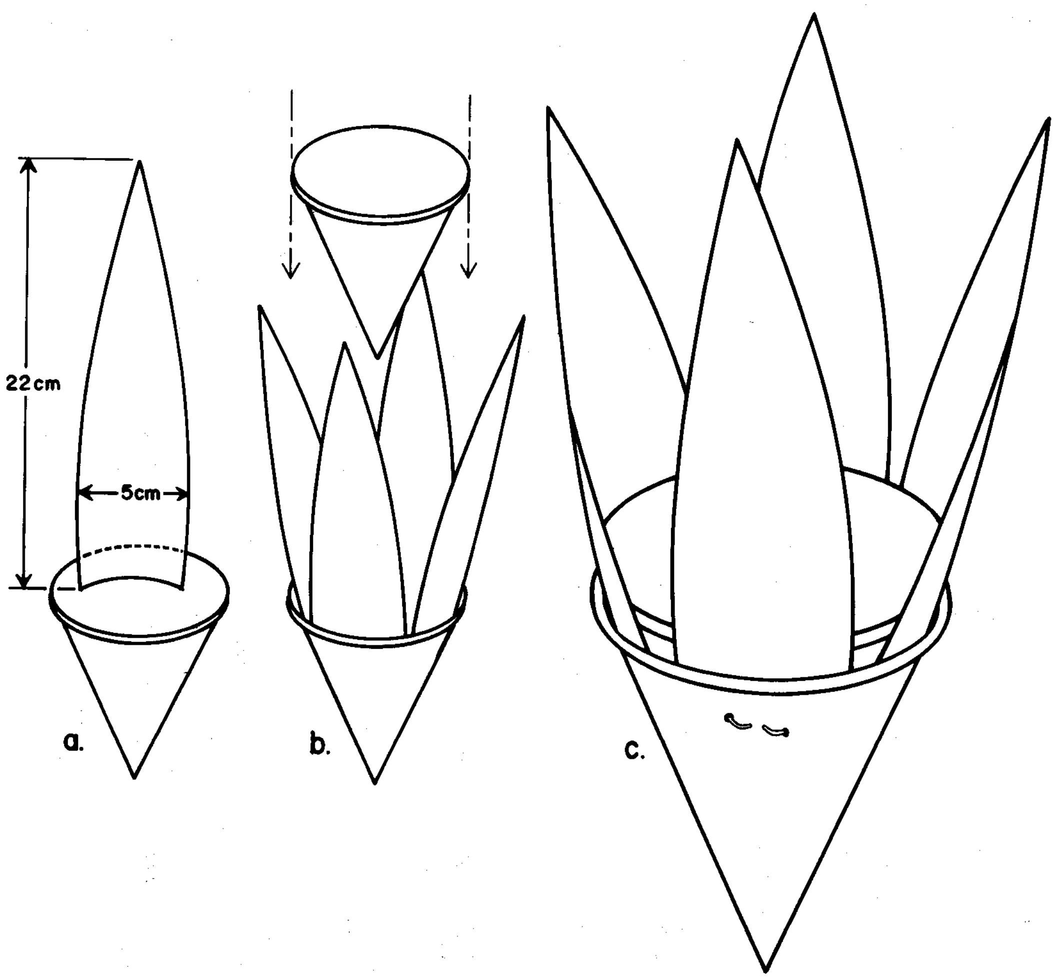

Fig. 1. Construction of an artificial bromeliad used in oviposition trials of caged mosquitoes; 1(a) a template was made by tracing a bromeliad leaf, and the template was used in cutting artificial leaves from brown paper to fit within a conical waxed paper water cup; 1(b) the water cup was fitted with four artificial leaves held in place by a second water cup; 1(c) the cups were stapled together and the entire structure was sprayed with two coats of gloss enamel paint and dried for several weeks before use. The paint provided waterproofing and color.

oviposition trap at Vero Beach in December 1983. Adults were maintained in separate cages (width 65 cm × height 50 cm × depth 38 cm) in a constant temperature room at 27°C, > 90% RH, and L:D 12:12. In the light cycle, 4 overhead fluorescent tubes (Westinghouse F72T12/D) supplied light. In the dark cycle, 2 wall-mounted 3.5 W incandescent bulbs supplied light. The bulbs were fitted with cardboard shades to prevent direct light reaching the mosquito cages. Cages were supplied with vials of 10% sugar water with cotton wicks. Blood from a human hand was offered daily. The *Wyeomyia* and *Aedes* adults fed on blood during the light cycle, but the *Culex* would feed only during the dark cycle.

A model bromeliad was supported in a narrow-mouthed glass jar in each of the 4 corners of the cage, clockwise white, dark green, deep blue, black. The cone of each model bromeliad (Fig. 1) was filled to about 40% of its depth with an infusion water. For the *Aedes* and *Wyeomyia* the model bromeliad was left in place for 48 hr to receive eggs, but for *Culex* only 24 hr because of the likelihood of *Culex* eggs hatching within 2 days. For *Aedes* and *Wyeomyia* the infusion water was made from about 75 g of fresh leaves of the bromeliad *Tillandsia utriculata* Linn. blended into 750 ml of tap water and strained to remove fibers to yield a yellowish green liquid. For *Culex,* which did not respond in a preliminary trial to the *Tillandsia* leaf infusion, a hay infusion was used instead following the method of Frank and Lynn (1982) but with yellow and green food dye added until the color matched that of *Tillandsia* leaf extract.

The model bromeliads were removed from the cages for examination of eggs. Contents were decanted and rinsed into petri dishes for examination under a microscope. Because many *Aedes* eggs were attached to the inner wall of model bromeliads just above the water line, a

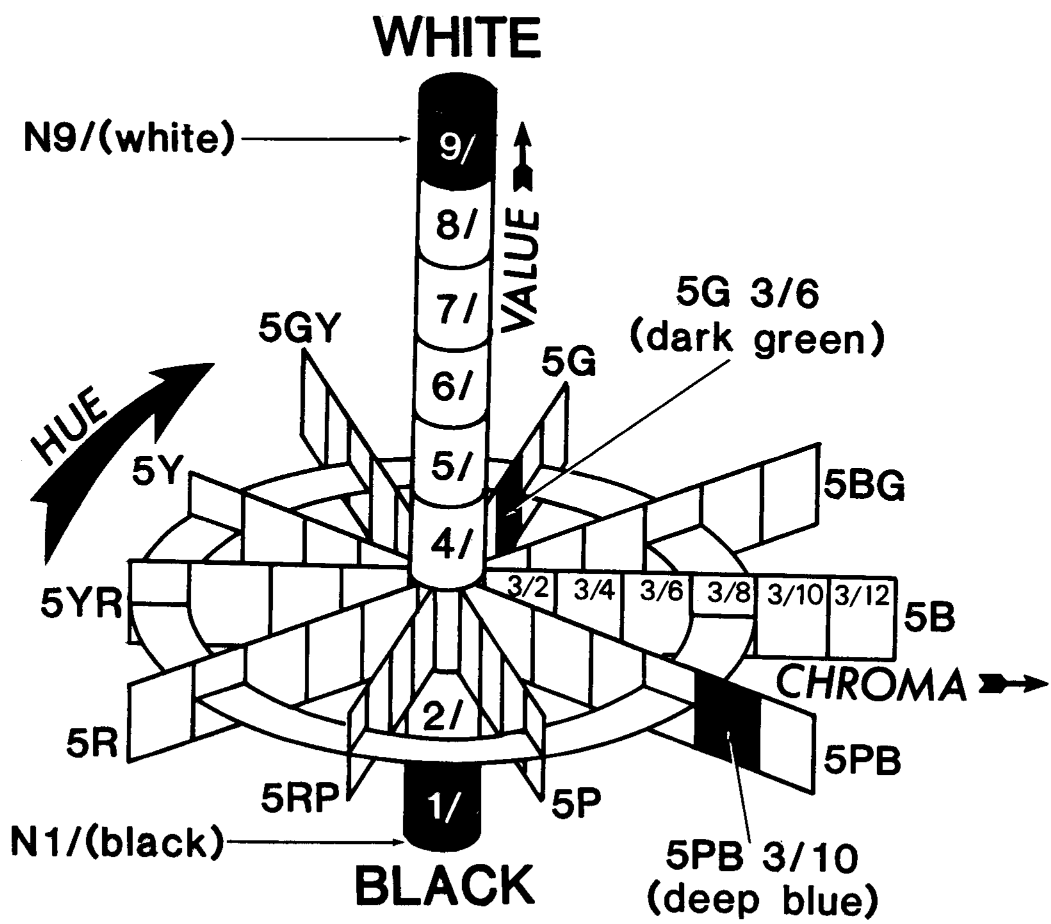

Fig. 2. The dimensions of the color solid as interpreted by Kelly and Judd (1955) and Munsell (1966). Positions of the two neutrals (black and white) and two colors (dark green and deep blue) used for artificial bromeliads are indicated.

single longitudinal cut was made in such walls so that the structure could be unfolded for examination under a microscope. Model bromeliads used for *Aedes* oviposition were thus destroyed during the sampling process, but those used for the other mosquitoes could be cleaned and reused. Eggs of *Aedes* and *Wyeomyia* were laid and counted singly, whereas those of *Culex* were laid and counted as entire rafts.

Model bromeliads were replaced in the cages in the same clockwise color order, but rotated one step clockwise. The experimental design thus ensured equal time of model bromeliads of each color in each corner of a cage to equilibrate possible bias. In total, eight sets of samples at 2-day intervals were taken of *Aedes* eggs and the same for each *Wyeomyia* species. Samples of *Culex* egg rafts were taken at 1-day intervals, and the number of egg rafts (but not of eggs) was much smaller than the number of eggs counted in experiments with the other species. In compensation, the experiment with *Culex* was continued for 16 days (as for the other mosquitoes) and then the results were pooled by 2-day intervals to yield eight sets of samples (as for the other mosquitoes).

The mosquitoes in each cage at any time were purposely of mixed ages and both sexes and an attempt was made to hold the number at roughly 100 per cage by adding a few newly-emerged adults each day to compensate for mortality. Mortality was due not merely to senescence but also to the drowning of some adults. Between-day variation in absolute numbers of eggs laid was smoothed by the 48 hr sampling interval (pooled to 48 hr in the case of *Culex*), but variation could not be eliminated. Therefore, within each of the eight samples for each species, numbers were first converted to percentages (percent of eggs in artificial bromeliad of each color), and then the STP non-parametric test was employed (Sokal and Rohlf 1969).

RESULTS

The results show striking differences between *Wyeomyia* and the other two genera (Table 1). Black model bromeliads [color value = 1] were preferred by *Ae. aegypti* to dark green and deep blue [color value = 3 for both] and to white [color value = 9], and were preferred to deep blue and white by *Cx. quinquefasciatus*. In contrast, very few *Wyeomyia* eggs were laid in the black models. Among the two *Wyeomyia* species, *Wy. mitchellii* alone showed a significant preference for dark green and white over deep blue.

DISCUSSION

The results provide an uncomplicated explanation for the relative abundance of preimaginal *Wyeomyia* in bromeliad leaf axils and relative scarcity of *Ae. aegypti* and *Cx. quinquefasciatus*. The color value of bromeliad leaves in nature [value = 5 or higher on Munsell (1966) scale] is within the range [value range = 3 to 9] attractive to ovipositing *Wyeomyia* mosquitoes but is too high to be very attractive to ovipositing *Ae. aegypti*, at least when alternative oviposition sites are available. Ovipositing *Cx. quinquefasciatus* were significantly more attracted to low color values [value = 1] than to high [value = 9], with dark green [value = 3] being of intermediate attractiveness, so the color value of bromeliad leaves in nature [value = 5 or higher] is not highly attractive. Additionally, ovipositing *Cx. quinquefascuatus* are attracted to oviposition sites containing a rich infusion of organic material (Frank and Lynn 1982). Rich organic infusions are rare in bromeliad leaf axils and were seen in *B. pyramidalis* axils only as a result of human activities: (1) a bed of *B. pyramidalis* received grass clippings ejected from a lawn mower, (2) a bed of *B. pyramidalis* was mulched to promote growth (personal observations).

After physiological experiments with *Ae. aegypti*, Snow (1971) thought that published observations on the color responses of mosquitoes could be explained by inferring a peak of sensitivity in the yellow-green range and perhaps a second peak in ultra-violet. The experiments reported here are in the area of behavioral ecology, not of physiology, and they neither support nor dispute Snow's (1971) conclusions.

Table 1. Distribution of eggs by caged mosquitoes among artificial bromeliads of two colors (deep blue, dark green) and two neutrals (black, white). Results are expressed as total number of eggs laid (egg rafts for *Culex*) during 8 replicated trials. Within each column, numbers followed by the same letter indicate no significant difference at the 5% level (STP test on data of eight separate trials).

Color of artificial bromeliad	Eggs *Wyeomyia mitchellii*	Eggs *Wyeomyia vanduzeei*	Eggs *Aedes aegypti*	Egg rafts *Culex quinquefasciatus*
Black	6 a	37 a	1522 a	33a
Deep blue	166 b	452 b	328 b	10 b
Dark green	777 c	690 b	394 b	18 a,b
White	1464 c	1120 b	73 b	11 b

They show that there are significant interspecific differences in response of females of four mosquito species to color value of oviposition site. For *Wy. mitchelli* alone, there was also a significant difference in response to one or more other dimensions of color (hue or chroma). The STP test of significance used is very conservative. It is intended to pursue the question of color response of the two *Wyeomyia* species in finer detail.

Use of a *Tillandsia* leaf extract was predicated because *Wy. smithii* preferred to oviposit in an extract of *Sarracenia* leaves when plain water was offered as an alternative (Istock et al. 1983). It is yet unknown whether *Tillandsia* leaf extract is preferred to plain water by *Wy. vanduzeei* and *Wy. mitchellii*, so this is a subject for future investigation. Development of the artificial bromeliad also will permit investigation of the shape of oviposition site preferred or required by gravid females of the two *Wyeomyia* species.

ACKNOWLEDGMENTS

I thank N. Hale (Baltimore), J. F. Day, J. R. Linley, L. P. Lounibos and G. F. O'Meara (Vero Beach), for critical comments on the manuscript of this paper.

References Cited

Bates, M. 1940. Oviposition experiments with anopheline mosquitoes. Am. J. Trop. Med. 20:568–583.

Beckel, W. E. 1955. Oviposition site preference of *Aedes* mosquitoes (Culicidae) in the laboratory. Mosq. News 15:224–228.

Benzing, D. H. 1980. The biology of the bromeliads. Mad River Press, Eureka, CA. xvi + 305 pp.

Fay, R. W. and D. A. Eliason. 1966. A preferred oviposition site as a surveillance method for *Aedes aegypti*. Mosq. News 26:531–535.

Fay, R. W. and A. S. Perry. 1965. Laboratory studies of ovipositional preferences of *Aedes aegypti*. Mosq. News 25:276–281.

Frank, J. H. and H. C. Lynn. 1982. Standardizing oviposition traps for *Aedes aegypti* and *Culex quinquefasciatus:* time and medium. J. Fla. Anti-Mosq. Assoc. 53:22–27.

Hilburn, L. R., N. L. Willis and J. A. Seawright. 1983. An analysis of preference in the color of oviposition sites exhibited by female *Toxorhynchites r. rutilus* in the laboratory. Mosq. News 43:302–306.

Istock, C. A., K. Tanner and H. Zimmer. 1983. Habitat selection by the pitcher-plant mosquito, *Wyeomyia smithii:* behavioral and genetic aspects, pp. 191–204, *In:* J. H. Frank and L. P. Lounibos (eds.) Phytotelmata: terrestrial plants as hosts for aquatic insect communities. Plexus Publ., Medford, NJ. vii + 293 pp.

Kelly, K. L. and D. B. Judd. 1955. The ISCC-NBS method of designating colors and a dictionary of color names. National Bureau of Standards, Washington, D.C., Circular 553 with supplement (standard sample no. 2106: ISCC-NBS color-name charts illustrated with centroid colors).

Lund, H. O. 1942. Studies on the choice of a medium for oviposition by *Anopheles quadrimaculatus* Say. J. Natl. Malaria Soc. 1:101–111.

McDaniel, I. N., M. D. Z. Bentley, H. P. Lee and M. Yatagai. 1976. Effects of color and larval-produced oviposition attractants on oviposition of *Aedes triseriatus*. Environ. Entomol. 5:553–556.

Munsell. 1966. Munsell book of color; glossy finish collection. Munsell Color Co. Inc., Baltimore, MD. 1966 edition.

O'Gower, A. K. 1963. Environmental stimuli and the oviposition behaviour of *Aedes aegypti* var. *queenslandensis* Theobald (Diptera, Culicidae). Anim. Behav. 11:189–197.

Slaff, M. E., J. J. Reilly and W. J. Crans. 1975. Colonization of the predaceous mosquito *Toxorhynchites rutilus septentrionalis* (Dyar & Knab). Proc. N.J. Mosq. Control Assoc. 62:146–148.

Snow, W. F. 1971. The spectral sensitivity of *Aedes aegypti* (L.) at oviposition. Bull. Entomol. Res. 60:683–696.

Sokal, R. R. and F. J. Rohlf. 1969. Biometry. Freeman, San Francisco, CA. xxi + 776 pp.

Williams, R. E. 1962. Effect of coloring oviposition media with regard to the mosquito *Aedes triseriatus* (Say). J. Parasitol. 48:919–925.

Yap, H. H. and A. E. S. Foo 1984. Laboratory studies on the oviposition site preference of *Toxorhynchites splendens* (Diptera: Culicidae). J. Med. Entomol. 21:183–187.