



Exposure of a diurnal mosquito vector to floral mimics: Foraging responses, feeding patterns, and significance for sugar bait technology

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ABSTRACT

Food location by mosquitoes is mediated by resource-derived olfactory and visual signals. Smell sensation is intermittent and dependent on the environment, whereas visual signals are continual and precede olfactory cues. Success of mosquito bait technology, where olfactory cues are used for attraction, is being impeded by reduced attractiveness. Despite proof that mosquitoes respond to colored objects, including those mimicking floral shape, and that they can discriminate among flowers, the impacts of artificial flowers on foraging remain unexplored. Using artificial flowers with sugar rewards, we examined the foraging responses of *Aedes aegypti* to various colors in equal choice bioassays. Starved adults were exposed to single flowers with petals of a given color (Single Blue Flowers [SBFs]; Single Red Flowers [SRFs]; Single Yellow Flowers [SYFs]; Single Pink Flowers [SPIFs]; and Single Purple Flowers [SPFs]) and two others with white petals (SWFs). Discrepancies in response time, visitation, feeding, and resting of both sexes were compared between colored flowers and SWFs. *Ae. aegypti* exhibited shorter response times to colored flowers compared to SWFs, but this behavior was mostly seen for SBFs or SYFs in females, and SRFs, SYFs, SPIFs, or SPFs in males. When provided an option to land on colored flowers and SWFs, female visitation occurred at high rates on SBFs, SRFs, SYFs, SPIFs, and SPFs; for males, this preference for colored flowers was seen to a lesser degree on SBF and SPIFs. Both sexes exhibited preference for colored flowers as sugar sources, but with different patterns: SPIFs, SRFs, SYFs, and SPFs for females; SYFs, SPFs, SPIFs and SRFs for males. Females preferentially rested on colored flowers when in competition with SWFs, but this preference was more pronounced for SPFs, SRFs, and SBFs. Males exhibited an increased preference for SRFs, SPFs, and SYFs as resting sites. Our results indicated the attraction of *Ae. aegypti* to rewarding artificial flowers, in some cases in ways similar to live flowering plants. The discovery that both male and female *Ae. aegypti* can feed on nectar mimics held by artificial flowers opens new avenues for improving sugar bait technology and for developing new attract-and-kill devices.

1. Introduction

For most key insect pollinators—i.e., bees and butterflies (Ballantyne et al., 2017; Ghazanfar et al., 2016)—the two most usual floral rewards are pollen and nectar (Russell and Papaj, 2016). Adult mosquitoes, which typically utilize nectar and plant juices as their main energy sources (Gary and Foster, 2004),

frequently interact with plant floral systems (Clements, 1992; Foster and Takken, 2004) throughout their lives (Xue and Barnard, 2003; Foster, 1995). This inherent association with flowering plants has been explored for the generation of novel methods of mosquito control (Lea, 1965; Allan, 2011). Sugar bait technology has been used in the search for sustainable control of mosquito-borne diseases (Fiorenzano et al., 2017). In particular ATSB (Attractive

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Toxic Sugar Bait) offers a promising “attract-and-kill” strategy for mosquito management (Beier et al., 2012; Junnila et al., 2015), fueled by the results of a number of successful field trials (Müller and Schlein, 2006; Müller et al., 2008, 2010a,b).

The technique for ATSB is based on a combination of sugar-based food source, an oral insecticide, and an olfaction stimulant to attract and kill mosquitoes (Revay et al., 2014) via spraying on plants or suspension in portable bait stations outdoors (Beier et al., 2012). Its efficacy is mainly dependent on the persistence of the attractant (Revay et al., 2014). As the attractants in ATSB solutions are mostly derived from plants and the environment contains large amounts of natural sugars and attracting sources (Fiorenzano et al., 2017), Schlein and Müller (2010) argued that the use of ATSB may be impractical in habitats with abundant attractive and competing flowers. Another problem is that, in the field, smell would move with the wind in intermittent packets of high concentration interspersed with background air (Murlis et al., 2000). The intermittent structure of natural plumes will cause adult mosquitoes to experience a brief puff of odor, and then nothing for seconds or even minutes as evidenced in many other animals (Murlis et al., 2000, 1992; Riffell et al., 2008). Rain and high temperatures in tropical areas are additional factors that could impede the effectiveness of ATSB. There have been many recent studies regarding the use of diverse scents to identify powerful attractants (Müller et al., 2010a,b; Fiorenzano et al., 2017). To overcome the potential insufficient attractiveness of ATSB, some entomologists have suggested the use of dyed sugar solutions (Qualls et al., 2015). When dyes are exposed to sun or rain, they can easily fade or dissolve and release unwanted toxicants into the environment. The idea behind Qualls’s proposition is that a visual attractant is necessary in sugar bait strategy.

In addition to olfactory cues, many other cues are potentially available to guide mosquito navigation. In particular, vision plays a crucial role in adult mosquito biology, including location of food sources (Allan et al., 1987). Host visual signatures—patterns, contrast, and color—have been reported to be key factors affecting the foraging of some insects including (Allan et al., 1987) including *Ae. aegypti* (Cardé, 2015). Many groups of insects are attracted to specific colors i.e., bees and mosquitoes (Packard, 1903; Hempel de Ibarra et al., 2015), which has long been exploited to develop highly effective trapping devices, with one of the most popular designs being the sticky trap. Yellow sticky traps—rigid material of a particular color coated with a sticky substance (Yee, 2011)—have been used successfully for monitoring and controlling insect pests (Byrne et al., 1986; Lu et al., 2012) including dengue vectors (Roslan et al., 2017). This technology is based on the natural attractiveness of yellow to many insects (Shimoda and Honda, 2013). Most insects that respond to yellow traps are active during the day (Shimoda and Honda, 2013). A special characteristic of these insects is that they possess apposition eyes (Hempel de Ibarra et al., 2015). This type of eye confers trichromatic color vision with high sensitivity (Sarkar and Theuwissen, 2013). Trichromatic insects, including mosquitoes, can perceive a complete spectrum of colors and discriminate between single colors and mixtures of colors (Matthews and Matthews, 2010), with edges being particularly noticeable (Wood and Wright, 1968; Browne and Bennett, 1981; Bidlingmayer, 1994).

Mosquito dispersal, oviposition, appetitive flight, resting, and host or sugar location are all governed by vision to some extent (Burkett and Butler, 2005). During foraging, olfactory cues follow, but do not precede, visual orientation (da Silva et al., 2015; Bezerra-Silva et al., 2016). Vision plays a larger role in guiding mosquito flight paths before and during appetitive flights (Bidlingmayer, 1994). Odor has effects over a short range and are used more to evaluate the suitability rather than the location of a stimulus (Bidlingmayer, 1994). Visual cues, in particular color, are crucial to the behaviors of dengue mosquitoes, which are diurnal (Hoel et al., 2011; da Silva et al., 2015; Bezerra-Silva et al., 2016). Although some *Aedes* species are known to be vectors for pollen transmission (Gorham, 1976), dengue vectors have been reported to also use flowering plants as an indicator of breeding opportunities. Davis et al. (2016) investigated oviposition preference of *Ae. albopictus* relative to flower presence and found that females prefer sites near butterfly bushes over sites without flow-

priori as sugar sources (Gouagna et al., 2010), there is evidence that dengue vectors do not acquire sugar meals from all flowering plants. In a field study to assess the attraction of *Ae. albopictus* to flowers and other plant materials, Müller et al. (2011) tested several flowering ornamental and wild plants. Significantly high levels of attraction were observed only for four of six ornamental flowers and four of eleven wild flowers. Abdel-Malek and Baldwin (1961) reported that *Ae. aegypti* fed on only three of 24 plant species offered to them, and suggested that this mosquito displays a discriminative preference among flowering plants.

Mosquitoes and bees are nectar-feeders with apposition eyes (Jeon, 2006). Artificial flowers have been successfully used to attract bees (Keasar, 2000), and many groups have studied how floral properties influence pollinator foraging behavior, including cognition, using artificial flowers (Chittka and Thomson, 1997; Gegear and Laverty, 2005; Ohashi and Thomson, 2009; Muth et al., 2015; Russell and Papaj, 2016). Essenberg (2015) reported that using such artificial flowers allows pollinators to forage for long periods of time under experimental conditions. Mosquitoes interact with plants for resting (Burkett-Cadena et al., 2008) or to acquire sugar from nectar (Clements, 1992; Foster, 1995; Foster and Takken, 2004). There have been a number of reports of successful attraction of adult dengue vectors by visual cues that were not associated with any olfactory component. For example, Brett (1938) demonstrated the presence of color preference in *Ae. aegypti* by exposing adults to cloths of different colors. Muir et al. (1992a) reported that stationary objects of solid colors are highly attractive to males and females of *Ae. aegypti*. This mosquito was found to be highly responsive to red-colored sticky cardboard (Kay et al., 2000). Despite evidence that dengue vectors respond to colored objects and that they exhibit discriminative preferences among flowers, it remains unclear whether artificial flowers can affect their foraging behavior. The present study was performed to examine whether *Ae. aegypti* is attracted to artificial flowers of various colors that do not offer any odor.

2. Materials and methods

2.1. Mosquito colony and experimental subjects

A sub-colony of *Ae. aegypti* was produced from a colony maintained under controlled conditions (26 °C–28 °C, 75%–85% relative humidity, and 14:10h L:D photoperiod) at the Entomology Unit of the Faculty of Resource Science and Technology (Universiti Malaysia Sarawak, Kota Samarahan). Replicates of 100–200 larvae were reared in plastic trays (As One Corporation, Osaka, Japan) containing 1L of tap water and fed every 2 days a diet consisting of cat food pellets (ProDiet Cat Food, Malaysia). Adults were held in cages (30 × 30 × 30 cm, BugDorm; MegaView Science Co., Ltd., Taichung, Taiwan) with continuous access to 10% sucrose suspension. About 3–4 days after emergence, females were allowed to take blood meals from two retrained hamsters during a 30-minute feeding period (Approval from Biological Research Ethics Committee at University Malaysia Sarawak). Eggs collected after 3 days of blood digestion and dried under laboratory conditions were kept as a colony stock. To obtain experimental adults, egg specimens from the colony stock were submerged in tap water and four replicates of larval populations each with 200 newly hatched larvae were reared as outlined above. Larvae were supplied 0.15g of the diet outlined above every 2 days and the rearing medium was replaced with fresh medium before the third food supply. Pupae were collected in 250-mL plastic vials containing 10 mL of water, and moved to mosquito cages equipped with a permanent 10% sugar suspension. Females (4–6 days old) and males (3–4 days old) starved for 1 day were used as experimental adults.

2.2. Experimental artificial flowers

High-quality artificial tulips (Super Save Co., Ltd., Kuching, Malaysia) were used in this study. Five petal colors previously reported to be attractive to mosquitoes or key pollinators were selected: yellow, purple, pink, red, blue, and white. Yellow color is known to be enticing to several insect groups (Byrne et al., 1986; Lu et al., 2012), including *Aedines* (Brett, 1938; Browne and

Bennett, 1981), a phenomenon that has been exploited to develop yellow sticky traps (Shimoda and Honda, 2013). The yellowish flowers of the wild ornamental plants *Prosopis farcta* and *Ziziphus spina-christi* were reported to be attractive to *Ae. albopictus* (Müller et al., 2011). The purple flowering butterfly bush (*Buddleja davidii*) (Davis et al., 2016) and the purple flowers of the chasteberry *Vitex agnus-castus* (Müller et al., 2011) were reported to be inviting signals for oviposition site seeking. *Ae. albopictus* were attracted to different colored plants including *Tamarix chinensis*, a plant with pink flowers (Müller et al., 2011). Red and blue objects were shown to be highly attractive to *Aedes* mosquitoes, including *Ae. aegypti* (Brett, 1938; Kellogg and Wright, 1962; Browne and Bennett, 1981). Green-tinted red flowers of *Ceratonia siliqua* were shown

to be appealing to *Ae. albopictus* (Müller et al., 2011). This latter dengue vector was reported to be highly responsive to white flowers of *Polygonum baldchuanicum* and *Polygonum equisetiforme* (Müller et al., 2011). Fig. 1 shows the different artificial flowers used in this study.

2.3. Flower materials

Single flowers of the different selected colors—blue, red, yellow, pink, purple, and white—were tested in this study. Each single flower had a peduncle, a receptacle, four sepals, and five petals. For all test flowers, the first three parts (peduncle, receptacle, and sepals) were green and the color of the petal was

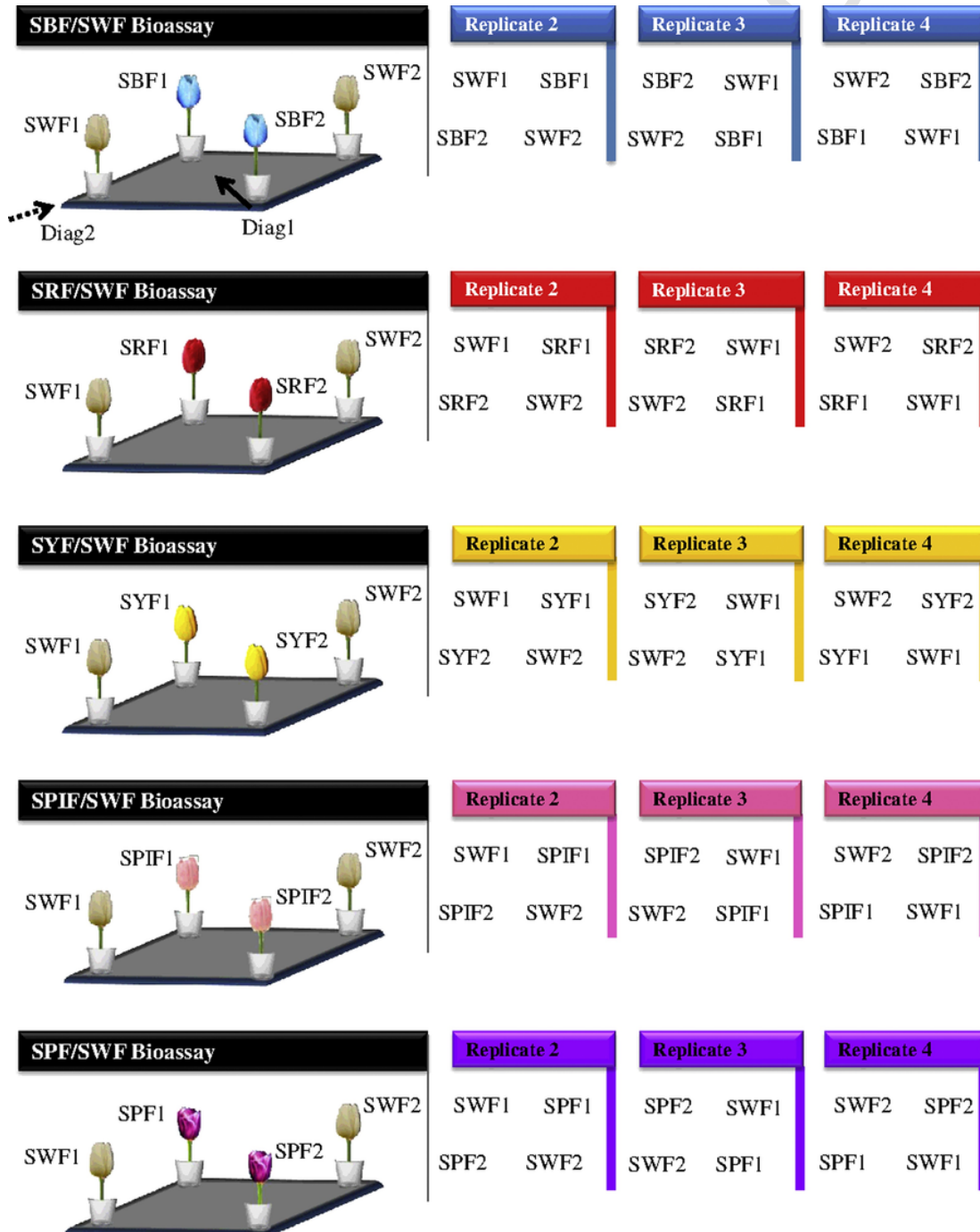


Fig. 1. Foraging bioassay design. The four flowers were held in the square polystyrene block that occupied the BugDorm cage. The flowers were positioned equidistantly from each other. A replicate consisted of one arrangement of the four flowers on the polystyrene block. In replicate 1, the two test flowers were placed on the diagonal 1—Diag1 (plain arrow) and the SWF pair (control) on diagonal 2—Diag2 (dotted arrow).

considered as the color of the flower. All experimental single flowers were equipped with a cotton wick (1 cm²) soaked with 2 mL of 10% sucrose solution. The cotton was placed inside the flower at the point of connection of petals. The single blue, red, yellow, pink, purple, and white flowers were labeled SBF, SRF, SYF, SPIF, SPF, and SWF, respectively.

2.4. Experimental features

All experiments were conducted according to the experimental design of Satho et al. (2015), but with slight modifications. The observation unit (OB) consisted of a cage (30×30×30 cm) with a square of polystyrene (side length = 27 cm) placed at the bottom center and holding four flowers at the four corners (Fig. 1). Each experimental flower was singly placed in a cubic polystyrene section to hold the peduncle and was equipped with a cotton wick (1 cm²) soaked with 2 mL of 10% sucrose solution that acted as a nectar source mimic. The cotton was placed inside the flower at the center (point of connection of petals). To avoid any potential position bias, all experiments involving single flowers were performed in a two-choice comparative test. In this case, each test flower and the control flower (SWF) were replicated four or eight times via a clockwise replication strategy (Satho et al., 2015) where a replicate will correspond to one arrangement of the four flowers on the square.

2.5. Bioassays

To determine whether SBF can influence the foraging behaviors of *Ae. aegypti*, such as response time, visitation, feeding, and resting, 4–6-day-old females were released into a cage (30×30×30 cm; Bug-Dorm) with an OB with four flowers: (a) SBF1; (b) SWF1; (c) SBF2; and (d) SWF2. Similarly, 3–5-day-old males were placed in another cage, and treated and observed in the same way. On the same or different days, three extra replicates of each of the two treatments (2 SBFs + 2 SWFs + 10 females, and 2 SBFs + 2 SWFs + 10 males) were set up and watched as described above for the two first bioassays. The same number of females or males, settings, procedures, and OB replicates reported for SBF were also used for (i) SRF, (ii) SYF, (iii) SPIF, and (iv) SPF. In all bioassays, immediately upon release of the 10 mosquito adults, an observer began noting the time to first visit, the numbers of visits, feeding, and resting events for each flower and within 30 min as described elsewhere (Dieng et al., 2017). All observations were carried out between 12:00 and 16:00 under controlled laboratory conditions (26 °C–28 °C, 75%–85% relative humidity, and 14:10 h L:D photoperiod).

2.6. Data collection and statistical analysis

In all bioassays, the time to first visit attempt by any one of the 10 females or males on any flower was monitored immediately after the collective release into the cage using a stopwatch. We considered a visit attempt as when an adult landed on a flower. The time between release and visit attempt was recorded for each cage replicate of each sex and test flower. In each case, the mean (\pm SE) of these time lengths (in seconds) was taken as a measure of response time, as reported elsewhere (Dieng et al., 2017). Any landing where the individual remained on the flower for at least 5 s was considered as a visit. The numbers of visits to a given flower were totaled and the percentages of female or male visits were calculated as follows: (number of visits to a flower)/(total number of visits to flowers within 30 min)×100. A feeding event was defined as contact between the mouthparts (stylet) and the cotton pad lasting for 15–20 s. The numbers of feeding events on each experimental flower were summed and percentages computed as described elsewhere (Dieng et al., 2016): (number of feeding events on a flower)/(total number of feeding events on all flowers in a given bioassay and within 30 min)×100. A resting event was considered as any landing where the individual remained on the flower for at least 20 s. The numbers of resting events on each test flower or SWF were used to determine resting percentages as: (number of resting events on a flower)/(total number of resting events on all flowers in a given bioassay in

30 min)×100. Visitation, feeding, and resting patterns on test and control flowers were characterized based on percentages. The differences in response time, visitation, feeding, and resting between test and control flowers were compared using the non-parametric Mann–Whitney *U* test with Social Science Statistics (<http://www.socscistatistics.com/tests/mannwhitney>). In all analyses, $p < 0.05$ was taken to indicate statistical significance.

3. Results

3.1. Patterns of female response time to flowers

In the presence of paired “SBF and SWF”, “SRF and SWF”, “SYF and SWF”, “SPIF and SWF” or “SPF and SWF”, no significant differences in female response time to test and control flowers were observed (Mann–Whitney *U* test, $p > 0.05$; Fig. 2A).

In the presence of two SWFs and two SBFs, *Ae. aegypti* males tended to visit earlier the first floral pair (350.28±124.9 s) than the second flower set (687.20±237.13 s), but the difference was insignificant (Mann–Whitney *U* test $z = 0.8944$, $p = 0.1867$). When paired SWFs (886.7±281.18 s) was in balanced competition with two SRFs (247.74±130.23 s), the mean response time of *Ae. aegypti* males was shorter to the latter pair than the first (Mann–Whitney *U* test $z = 19$, $p = 0.0282$); a similar pattern was observed when comparing response time of such males when exposed to a SWF pair (437.62±93.44 s) and a SYP pair (131.60±32.24 s) (Mann–Whitney *U* test $z = -2.8877$, $p = 0.0019$). In the presence of two SPIFs and paired SWFs, the mean male response time to the first pair (280.37±93.65 s) did not differ substantially with that toward SWFs (372.62±139.78 s) (Mann–Whitney *U* test $z = -0.1575$, $p = 0.4364$). An alike pattern of response was obtained when *Ae. aegypti* males were exposed to a SPF and SWF pairs (227.57±82.57 s vs. 399.00±92.35 s; Mann–Whitney *U* test $z = -1.4616$, $p = 0.0721$) (Fig. 2B).

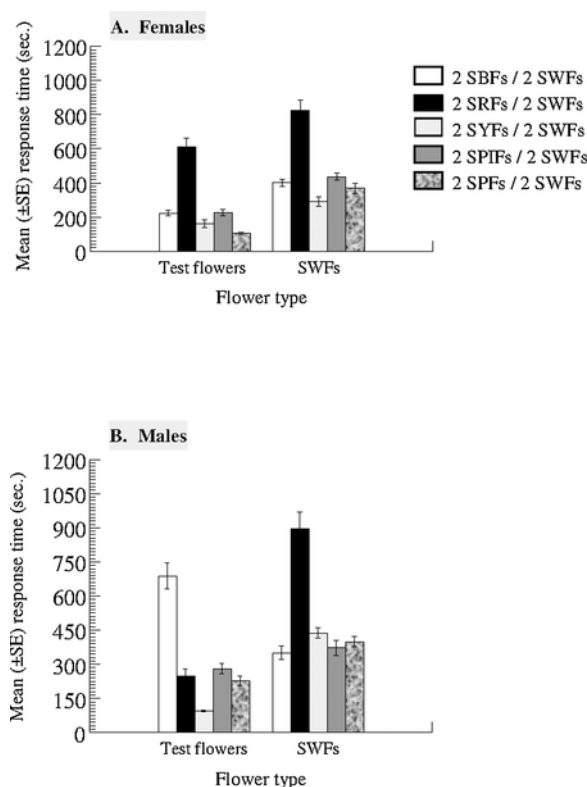


Fig. 2. Response times to flowers by *Ae. aegypti* females (A) and males (B) when provided equal chances to take sugar meals from two flowers of a given color (SBFs, SRFs, SYFs, SPIFs, or SPFs) and two others with white petals (SWFs).

3.2. Visitation preferences to colored flowers

When the SBF pair was in balanced competition with SWFs, 64.47% (49/76) of the total visits by *Ae. aegypti* females occurred at the first flower type. The mean number of visits at SBFs (9.80 ± 2.67) was greater than that at SWFs (2.45 ± 0.76) (Mann–Whitney *U* test $z = -2.32241$, $p = 0.010$). When there were four flowers, most visits by *Ae. aegypti* females took place at the SRF pair (76.66%, 27/30) rather than the SWF pair (23.34%, 7/30), with the mean at the first pair (2.87 ± 0.51) being higher than that at the second pair (0.87 ± 0.47) (Mann–Whitney *U* test $z = 2.36297$, $p = 0.009$). When two SYFs and two SWFs were present, 67.80% (40/59) of the total visits by females were at the first pair at a mean of 0.87 ± 0.47 ; these values were 32.20% and 2.37 ± 0.77 visits at the SWF pair, respectively. The rate of visits tended to be higher at SYFs, but both flower types were enticing to female *Ae. aegypti* (Mann–Whitney *U* test $z = 1.31276$, $p = 0.0951$). When there were equal opportunities, the SPIF pair received 38 visits (67.86%) by female *Ae. aegypti* vs. 18 visits (32.14%) at the SWF pair. The mean rate of visits at this latter pair (2.25 ± 0.52) was less than that recorded at the SPIF pair (4.75 ± 0.70) (Mann–Whitney *U* test $z = 2.25795$, $p = 0.0119$). When *Ae. aegypti* females were exposed to two SPFs and two SWFs, they performed 86 visits of which 82.59% (71/86) were on the first pair and 17.44% on the competing pair. The mean rate of visits at SPFs (8.87 ± 3.23) was far greater than that at the SWF pair (1.87 ± 0.66) (Mann–Whitney *U* test $z = 2.62553$, $p = 0.0042$) (Fig. 3A).

When *Ae. aegypti* males were presented with two SBFs and one SWF pair, 39.51% (32/81) of their visits occurred at the first flower type compared to 60.5% at the other type. When SRF was in equitable competition with SWF, visits by *Ae. aegypti* males occurred mostly at the first flower type (87.84%; 65/74). The mean rate of visits at SWF (1.12 ± 0.44) was appreciably lower than that at the SRF pair (8.12 ± 2.72) (Mann–Whitney *U* test $z = 2.62553$, $p = 0.004$). When two pairs of flowers were present, *Ae. aegypti* males tended to visit SYF (64.62%; 42/64) more often than SWF (35.38%). However, the mean rate of visits at the SYF pair (5.25 ± 1.19 visits) was not different from that at the SWF (2.87 ± 1.12 visits) (Mann–Whitney *U* test $z = 1.57532$, $p = 0.05705$). SPIF and SWF pairs were essentially equally attractive to *Ae. aegypti* males when both flower types were in equitable competition; 44.54%

(53/119) of the total visits were at SPIF and 55.46% at SWF. When SPF and SWF pairs were in equitable competition, 75.86% (22/29) of the total visits were on the first pair (Fig. 3B).

3.3. Flowers and sugar feeding patterns

In the two-choice bioassay related to blue-colored flowers, both SBF and SWF were used equally by *Ae. aegypti* females. Half of the total feeding events occurred on SBFs and the other on SWFs. Under the same choice conditions, males fed on sugar 90 times, of which 57.90% were on the first flower type and 42.10% on the other floral entity. In the two-choice bioassay involving red-colored flowers, *Ae. aegypti* females fed on sugar 13 times, and 69.23% of these feeding events took place on SRFs vs. 30.64% on competing SWFs. When similar feeding opportunities were presented to males, sugar feeding activity on SRFs (79.16%) was 3.29 times that on SWFs. When provided with the same sugar acquisition options from two SYFs and two SWFs, *Ae. aegypti* females fed 2.40 times more often on the SYP source (70.59%) than on the SWP source (29.40%). A similar pattern of feeding preference was observed when males were exposed to two SRFs (62.85%) and two SWFs (37.15%). When given equal chances to feed on sugar from a pair of SPIFs and a pair of SWFs, the majority of sugar uptake events by *Ae. aegypti* females occurred on the first type of flower (66.67%), with a rate double that on competing flowers (33.33%). An identical pattern was also found for males, with sugar feeding activity on SPIFs (70.37%) 2.37 times that on SWFs. When provided with paired SPFs and two SWFs, sugar feeding occurred on both resources, and 87.73% of the meals came from SPFs vs. 12.27% from SWFs. Of the 13 sugar uptake events by male conspecifics exposed to similar choices, 69.23% occurred on SPFs and 30.77% on SWFs (Table 1).

3.4. Resting preferences on flowers

When two different pairs of flowers were available, *Ae. aegypti* females rested mostly on SBFs (76.62%; 16/21) rather than SWFs (23.80%). In the paired SRF/paired SWF bioassay, 95.24% (20/21) of the total resting events occurred at the first pair with only 4.76% at the other pair. The mean number of resting events at SRFs (2.50 ± 0.75) was significantly higher than that recorded on SWFs (0.12 ± 0.12) (Mann–Whitney *U* test $z = 3.09812$, $p = 0.0009$). When two SYFs and two SWFs were the only sites, 72.41% (21/29) of resting events took place at the SYF pair and 27.59% at the SWF pair with respective means of 2.82 ± 0.78 and 1.00 ± 0.46 that were significantly different (Mann–Whitney *U* test $z = 1.6278$, $p = 0.0515$). When there were identical chances to rest on two different floral pairs, *Ae. aegypti* females showed a preference for SPIFs (70.83%, 17/24) over SWFs (29.17%). The mean number of resting events at SPIFs was consistently higher than that at SWFs (2.12 ± 0.39 vs. 0.87 ± 0.39 ; Mann–Whitney *U* test $z = 1.94289$, $p = 0.0262$). In the presence of two SPFs

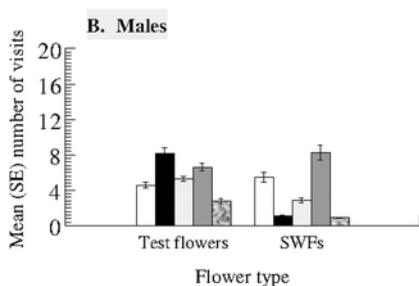
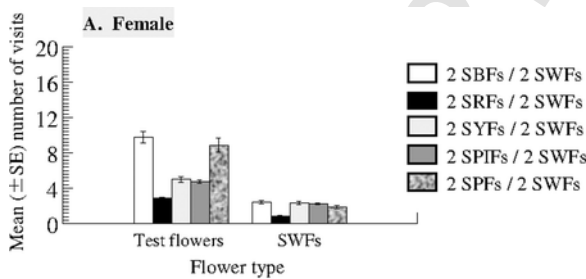


Fig. 3. Visitation of flowers by *Ae. aegypti* females (A) and males (B) when provided equal chances to land on two flowers of a given color (SBFs, SRFs, SYFs, SPIFs or SPFs) and two others with white petals (SWFs).

Table 1

Sugar uptake responses of *Ae. aegypti* adults when provided equal chances to take sugar meals from two flowers of a given color red single flowers (SBFs, SRFs, SYFs, SPIFs or SPFs) and two others with white petals (SWFs).

The four flowers	Total number of feeding events			
	Female		Male	
	Test flower	SWF	Test flower	SWF
SBF1, SBF2, SWF1, SWF2	9	9	8	11
SRF1, SRF2, SWF1, SWF2	9	4	19	5
SYF1, SYF2, SWF1, SWF2	12	5	22	13
SPIF1, SPIF 2, SWF1, SWF2	10	5	19	8
SPF1, SPF2, SWF1, SWF2	93	13	9	4

and the SWF pair, *Ae. aegypti* females visited both floral types. There were 41 resting events, of which 92.68% occurred at the first pair with the remainder at the other floral type. These resting events occurred with a greater mean at SPFs (4.75 ± 1.03 ; 0.37 ± 0.18 ; Mann–Whitney *U* test $z = 2.73055$, $p = 0.0031$) (Fig. 4A).

When *Ae. aegypti* males were presented with two SBFs and two SWFs, they showed similar numbers of resting events on the two types. When two different pairs of flowers were accessible, *Ae. aegypti* males exhibited a significant preference for resting on SRFs (100%, 43/43), completely ignoring the SWFs (Mann–Whitney *U* test $z = 3.30816$, $p = 0.0004$). When two SYFs were presented to *Ae. aegypti* males in the presence of two SWFs, 72.97% (27/37) of the total resting events were at the first pair. The mean number of resting events at SWFs (1.25 ± 0.62) tended to be lower than that seen at SYFs (3.37 ± 1.42), but the difference was not significant (Mann–Whitney *U* test $z = 1.62783$, $p = 0.0515$). When *Ae. aegypti* males were given a choice between two SPIFs and two SWFs, 60% (15/25) of resting events were at the first flower pair and 40% (10/25) were at the second set. With equivalent accessibility of SPF and SWF, *Ae. aegypti* males visited both floral pairs. Of the 25 resting events, 80% (20/25) went to the SPF pair and 20% to the SWFs. The mean resting occurrence tended to be higher at SPF sites (2.50 ± 0.92) than in the SWF environment (0.62 ± 0.18), but the difference was not statistically significant (Mann–Whitney *U* test $z = 1.68034$, $p = 0.0464$) (Fig. 4B).

4. Discussion

There is a great deal of research interest regarding the use of color to control insect pests (Diaz and Fereres, 2007; Ben-Yakir et al., 2012). Despite evidence that artificial flowers have strong impact on insect pollinator behavior (Ohashi and Thomson, 2009; Muth et al., 2015), that mosquitoes are attracted to checkered patterns mimicking floral shapes (Bernáth et al., 2016), and that some colored objects are attractive to dengue vectors (Brett, 1938; Muir et al., 1992; Tainchum et al., 2013), the impacts of artificial flowers on mosquito behavior have not been investigated. This was the first formal attempt to document the behavioral significance of colored flowers for a dengue vector. Specifically, we investigated the foraging and resting behaviors of both sexes of *Ae. aegypti* using choice bioassays involving artificial flowers of various

colors. We used white-colored flowers (SWF) as controls based on their previous use as a standard for color studies in insects (Menzel and Shmida, 1993), including dengue vectors (Brett, 1938).

It should be noted that there have been few studies on artificial flower–mosquito interactions and most of the research work on color attraction in mosquitoes has used colored objects (Brown, 1954; Browne and Bennett, 1981; Burkett and Butler, 2005). For example, Brett (1938) examined color preference in *Ae. aegypti* by exposing adults to different colored cloths, reported that black was most attractive, red was very attractive, blue was neutral, and yellow was less attractive. Stationary solid objects (Muir et al., 1992), sticky cardboard (Kay et al., 2000), and fabrics (Tainchum et al., 2013) have been reported to be highly attractive to both sexes of *Ae. aegypti*. Artificial flowers are generally made from various materials, including polyester fabric, painted linen, silk, wood, ribbons and cotton (Hunter, 2013). These studies taken together with the recent report from Bernáth et al. (2016) indicating increased attractiveness of floral-shaped objects paired with a sugar source strongly suggest that the artificial flower-mediated foraging behaviors seen in the present study were not artifacts.

When SPIFs were present in equal competition, they attracted *Ae. aegypti* females earlier than SWFs. Females tended to display a faster response to either SBFs or SYFs when they were in balanced competition with SWFs. In addition, SRFs, SYFs, SPIFs, and SPFs tended to attract males sooner than SWFs. These observations suggest that there is a preference, which has been documented in studies regarding live flowers. In the field, *Ae. aegypti* was reported to feed on only three of 24 accessible flowering plants (Abdel-Malek and Baldwin, 1961), indicating the existence of well-defined preferences. *Vitex agnus castus* (with purple flowers), *Tamarix chinensis* (with pink flowers), *Prosopis farcta* (with pinkish flowers), *Ziziphus spina christi* (with pinkish flowers), and *Prosopis farcta* (with pinkish flowers) have been reported to be highly attractive to both sexes of dengue vectors in the presence of white-colored flowers (*Anthemis pseudocotula*, *Conyza bonariensis*, and *Nerium oleander*), which were unattractive (Müller et al., 2011). Flowers of *Solidago canadensis*, which are yellow, were highly attractive as a sugar source for aedine mosquitoes (Ebrahimi, 2013). In the present study, we tested five floral colors, i.e., light blue, red, yellow, pink, and purple. Of these test colors, the shortest response time (for females) of about 2min 15s was observed with purple flowers (SPFs), which had the strongest color. Floral colorfulness advertises reward type and quantity for nectar-feeding insects (Giurfa et al., 1995; Menzel and Shmida, 1993; Binkenstein et al., 2016). Several groups have established variable degrees of linkage between floral color, nectar/sugar amounts, and insect visitation pattern. Raine and Chittka (2007) reported increased preference of bees for blue and purple flowers, and suggested that this was because such flowers are highly profitable in terms of nectar rewards and amounts of sugar. Zhang et al. (2017) examined whether color change affects the quantity of floral rewards in *Arnebia szechenyi*, the flowers of which are yellow. They found that floral rewards were markedly reduced and attractiveness declined when color vividness decreased. Another study (Culin, 1997) assessed the connections between flower color, nectar quality, and butterfly visitation intensity using thirteen cultivars with flower colors varying from white to dark purple. They reported significantly greater amounts of butterfly activity on the cultivars “Charming Summer” (with lavender-pink flowers), “Royal Red” (with deep pink flowers), “Pink Delight” (with pink flowers), and “Petite Plum” (with deep pink/purple flowers). They also reported increased visitation rates on varieties with high levels of nectar and sugar, especially red, pink, or lavender-pink flowers, and decreased visitation events on white or pale lavender flowers. In the present study, all flowers examined were similar in dimensions and differed only in the color of the petals. Culin (1997) tested cultivars, which are generally produced from the same wild plant for a given characteristic. Most of the test floral colors in the present study corresponded to the colors used by Culin (1997), and both sexes of *Ae. aegypti* visited colored flowers at increased rates (males/females: SBFs: 39.8%/64.4%; SRFs: 87.7%/76.6%; SYFs: 72.9%/67.8%; SPIFs: 44.5%/67.8%; SPFs: 75.8%/82.5%) when compared to SWFs (males/females: 12.1%/35.5%). All test (SBF, SRF, SYF, SPIF, and SPF) and control (SWF) flowers were equipped with a cotton pad soaked

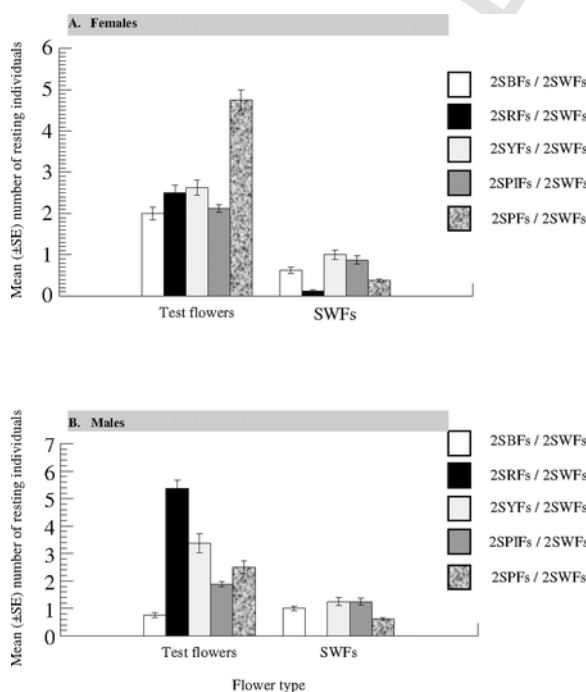


Fig. 4. Resting responses of *Ae. aegypti* females (A) and males (B) when exposed to two flowers of a given color (SBFs, SRFs, SYFs, SPIFs, or SPFs) and two others with white petals (SWFs).

in 10% sucrose solution that acted as a mimic of a natural nectar source, so potential sugar rewards were similar across all of the experimental flowers. All experimental mosquitoes were starved for 24 h prior to the tests. Bernáth et al. (2016) reported that, in the wild, floral sugars are ephemeral resources that can quickly become depleted. A high-quality nectar flower will tend to trigger an increased probability of visitation by many nectar-searching insects, which will create competition and decreased chance of sugar acquisition. Thus, quick access could be beneficial. The attractiveness of colored artificial flowers over SWFs may indicate that *Ae. aegypti* can discriminate between different flowers with respect to successful sugar acquisition. The observed shorter response time to SPFs is a possible move for successful sugar acquisition to combat hunger more quickly.

There was a clear connection between flower type and *Ae. aegypti* sugar uptake activity, with colored flowers showing greater attractiveness than competing SWFs. For females, except SBFs, feeding events ranged between 69.23% and 87.73% on colored flowers and varied between 12.27% and 30.73% on SWFs. A similar pattern of preference was noted for males, which however also tended to feed readily on SWFs. Differences in sugar feeding activity have often been associated with sugar resource availability, a link that has been well documented in mosquito vectors. Gouagna et al. (2010) reported that *Anopheles* species in different peri-domestic habitats favored some flowering plants over others despite their potential to serve a priori as nectar sources. Manda et al. (2007) reported that adults of the same mosquito genus land on plants only to acquire a sugar meal and that both sexes preferentially feed on plants with a high sugar content for increased fitness. Spencer et al. (2005) reported that the level of female sugar feeding was significantly greater during the dry, low-dengue transmission season, which they attributed to specific preferences for flowering plants that were abundant during this period. Davis et al. (2016) investigated oviposition preference in *Ae. albopictus* relative to flower presence, and found that females prefer sites near butterfly bush over sites without flowers for egg deposition. They suggested that gravid females preferred the flowers for sugar feeding opportunities for themselves and their offspring. Insects can differentiate between flower colors and exhibit preferences for those with increased sugar rewards (Raine and Chittka, 2007; Culin, 1997; Zhang et al., 2017). There is evidence that adult mosquitoes resort to visual cues that co-occur with vital nutritional resources (Bernáth et al., 2016). Considering the reports mentioned above, it is therefore plausible that *Ae. aegypti* adults (especially females) preferred colored flowers because they associate colorfulness with high sugar content and quality. It is also likely that the difference in attractiveness between the colored and white flowers (SWFs) was due to discrepancies in appearance and the natural behavior of *Ae. aegypti*—colorful flowers are more likely to be seen during daytime for a day-active mosquito. It is interesting to note that males tended to feed more on SWFs than females; this may have been because males need sugar more than females as a consequence of their fierce swarming activity (Foster and Hancock, 1994). Females use sugar to supplement blood feeding because they can gain energy from blood (Nayar and Sauerman, 1971; Edman et al., 1992; Scott et al., 1993).

Ae. aegypti adults also exhibited increased preference for colored flowers as resting sites. Floral colors elicited high female resting counts, but this effect was mostly seen with the strong colors—SPFs (92.62%), SRFs (95.24%), and SBFs (76.62%). In contrast, at SWF sites, resting incidence ranged between 4.67% and 29.17%. For males, SRFs (100%), SPFs (80%), and SYFs (72.97%) induced elevated resting rates. These observations were consistent with some previous reports. For example, Brett (1938) examined color preference in *Ae. aegypti* by exposing adults to different colored cloths, reported that black was most attractive, red very attractive, blue neutral, and yellow less attractive. Muir et al. (1992) reported that stationary objects of solid color were highly attractive to males and females of *Ae. aegypti*. This mosquito was found to be highly responsive to red-colored sticky cardboard (Kay et al., 2000). Gilbert and Gouck (1957) assessed landing preference in *Ae. aegypti* using various dye colors, and found that blue, yellow, and red were among the most attractive colors. Clearly, in the wild, resting on white petals will tend to result in death as resting individuals with a black body color on a white background are at elevated

risk of predation. In contrast, resting on dark-colored (blue, purple, red) petals would reduce the potential for predation.

5. Conclusions

ATSB technology has become widely adopted in the search for sustainable control of mosquito-borne diseases (Fiorenzano et al., 2017). The application of this technique consists of spraying on foliage (Stewart et al., 2013) or as bait stations (Fiorenzano et al., 2017). The effectiveness of ATSB depends heavily on attractant persistence (Revay et al., 2014). Although this approach had been successful in controlling mosquito populations (Khallaoune et al., 2013), there has been a great deal of research to identify new attractants (Schlein and Müller, 2008; Müller et al., 2010a,b, 2011). Several strategies have been applied or proposed to improve attraction, e.g., mixing dyes with sugar (Qualls et al., 2015) or using overripe fruit sources (Fiorenzano et al., 2017). Dengue and malaria are mostly prevalent in developing countries throughout the Tropics (Magalhães et al., 2014). Generally, such countries have high organic waste output (Hoorweg and Bhada-Tata, 2012; Pharino, 2017) and infrequent collections, resulting in long residence times for waste products (Banerjee et al., 2015). As such recurrent switches to competitive attracting sources are predicted during ATSB operations. Such countries are also characterized by constant high temperatures and lengthy rainy seasons. As exposure to high temperatures can cause solutions to evaporate rapidly, the sun may affect the efficacy of ATSB, and ATSB solutions sprayed on plants may experience splashing or dilution in areas with rainfall. ATSB technology is based on one aspect of plant–mosquito interactions, i.e., the location of sugar sources using odor sensation, which is intermittent (Murlis et al., 2000). In fact, food search decision in mosquitoes is initiated primarily using vision (Bidlingmayer, 1994), which is anterior to olfactory cues (da Silva et al., 2015; Bezerra-Silva et al., 2016). To address the issue of dengue vector control, Lenhart et al. (2008) has advocated that exploiting color has potential to contain the incidence of this disease. Color vision is unaffected by shifts in the intensity or color of the ambient light conditions (Johnsen et al., 2006; Kinoshita and Arikawa, 2014; Ogawa et al., 2015), or wind in contrast to odor (Murlis et al., 2000). The objective of the present study was to assess the foraging responses of *Ae. aegypti* to rewarding artificial flowers. Our results clearly demonstrated the ability of light- (SYFs and SPIFs) and dark-colored (SPFs, SBFs, and SRFs) artificial flowers to quickly and strongly attract mosquitoes of both sexes in manners similar to live flowering plants. The results also showed that test flowers of all colors acted as preferred resting sites for both males and females. This increased resting preference near colored flowers will tend to result in high cumulative sugar feeding. As dengue mosquitoes can resort to visual cues from artificial sources holding sugar and feed, exploring the attractiveness of artificial flowers to their foraging will improve sugar bait technology or attract-and-kill strategies. Incorporation of artificial flowers with persistent colors may not only enhance ATSB via long-lasting visual attraction, but also reduce insecticide use and related biodiversity loss.

Uncited references

Fernandes and Briegel (2005), Flacher et al. (2015), Nayar and Sauerman (1975), and Systat Software Inc. (2004).

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