Survival Value and Sugar Access of Four East African Plant Species Attractive to a Laboratory Strain of Sympatric *Anopheles gambiae* (Diptera: Culicidae)

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Abstract

Mosquitoes derive energy from plant sugar, thereby promoting survival and reproduction. Its survival value to females plays a key role in the vectorial capacity of mosquito populations. Previous olfactometry assays of responsiveness demonstrated that *Senna didymobotrya* Fresenius, *Parthenium hysterophorus*, *L. Senna occidentalis*, (L) and *Lantana camara* L were among the most attractive plants for the Mbita strain of *Anopheles gambiae* s.s. Giles in eastern Africa. Here, we provide experimental evidence that three of these four species also provide varying but substantial amounts of sugar for mosquito survival, whereas a fourth does not. Rank order of survival of both sexes of mosquitoes housed with these plants was as follows: *S. didymobotrya* was highest, followed by *S. occidentalis* and *L. camara*, whereas survival on *P. hysterophorus* was only slightly better than on only water. A positive control group, housed with 10% sucrose, survived well but fell significantly short of those with *S. didymobotrya*. A causal connection between survival and sugar availability was established by exposing mosquitoes to plants overnight, and then testing them for the presence and amount of undigested fructose. Fructose positivity was most frequent in those exposed to *L. camara*, whereas greatest amounts of fructose were obtained from *S. occidentalis* and *S. didymobotrya*. *Parthenium hysterophorus* scored lowest in both categories. We conclude that attractiveness and sugar availability are often, but not always, concordant. It remains unclear why *P. hysterophorus* should be attractive if it offers little sugar and does not prolong survival. Furthermore, the cause behind the superior survival benefit of *S. didymobotrya*, compared with 10% sucrose, is unknown.

Key words: *Anopheles gambiae*, mosquito, plant species, survival, sugar feeding

Adult females of most mosquito species ingest plant sugar and vertebrate blood, both foods contributing to flight, survival, and reproduction. Males rely entirely on plant sugar after larval reserves are exhausted (Yuval 1992, Foster 1995). The implications of sugar feeding for the vectorial capacity of a mosquito population are multiple, and both direct and indirect. For example, female survival beyond the pathogen's extrinsic-cycle duration is critical for successful pathogen transmission. Sugar also can promote fecundity in both sexes, which results in a higher population growth potential and therefore vector density (Mostoway and Foster 2004, Gary et al. 2009, Stone et al. 2009); it can also have a neutral or opposite effect (Gary and Foster 2001, Costero et al. 1998). The sugar is obtained chiefly from floral and extra-floral nectaries, sap, honeydew, and rotting or damaged fruit. *Anopheles gambiae* s.s. Giles (Diptera: Culicidae), a major vector of malaria in sub-Saharan Africa, appears

to be no exception to the utilization of plant sugar (Beier 1996, Gary and Foster 2004, Impoinvil et al. 2004, Manda et al. 2007a, Müller et al. 2010, Stone et al. 2012), though plant feeding by this and other members of the *An. gambiae* complex in the field is seldom seen and poorly documented.

The physiological or ecological explanation for the utilization of sugar by *An. gambiae* females, and also by *Aedes aegypti* L., despite being peridomestic species for which human blood appears to satisfy both their somatic and reproductive functions, is an issue not yet fully explored. The topic has been addressed elsewhere (Foster 1995, Gary and Foster 2001, Harrington et al. 2001, Foster and Takken 2004, Fernandes and Briegel 2005, Braks et al. 2006). *Anopheles gambiae*'s natural plant hosts are surmised principally from cage observations and staged field experiments or from measures of responsiveness to a plant's blend of volatile organic

compounds (VOCs; Manda et al. 2007a; Gouagna et al. 2010, 2014; Müller et al. 2010; Nyasembe et al. 2012; Nikbakhtzadeh et al. 2014), all of which indicate plant preferences. Plant-host use by mosquitoes is probably influenced through a combination of the plant's attractiveness, the accessibility of the plant's sugars and other nutrients, and the plant's relative abundance among sugar-bearing plant species in a plant community.

Selective plant attraction may have evolved to maximize the chance of obtaining sugar. The studies of Manda et al. (2007a,b) suggested that species preferences, as measured by relative frequency of *An. gambiae* landing and probing in large outdoor cages offering a broad choice of plant species, or by the composition of sugar mixtures ingested, corresponded to the plant's ability to promote longevity and sugar ingestion, with one odd exception. The olfactometer results of Gouagna et al. (2010, 2014) and combined behavioral observations of Nyasembe et al. (2012, 2014, 2015) from laboratory and field appear to support this correspondence between attractive VOCs and sugar availability. The exception reported by Manda et al. (2007b), *Parthenium hysterophorus* L., however, has been challenged by the investigations of Nyasembe et al. (2015), who in laboratory experiments found it to be both attractive and survival-promoting. We took the Manda study a step further by comparing the response to VOCs of many of the same sugar-accessible indigenous and exotic plant species common in eastern Africa and also one weakly attractive grass, *Dactylis glomerata* L. (Cyperales: Poaceae), apparently lacking external sugar (Nikbakhtzadeh et al. 2014). That olfactometer study confirmed the exceptional attractiveness of *P. hysterophorus*. In the present study, we have reexamined the ability of four of the plant species with the most attractive VOC emissions, including *P. hysterophorus*, both to provide sugar and to support longevity, to further test this attraction-sugar-survival relationship. This topic is linked to malaria suppression, because the availability of plant sugar might be constricted by selective plant elimination (Stone et al. 2012, B. Ebrahimi, unpublished data), and the volatiles making a plant attractive can be co-opted for toxic sugar baits (Müller et al. 2010).

Materials and Methods

Experimental Plants

Parthenium hysterophorus L. (Asterales: Asteraceae), *Lantana camara* L. (Lamiales: Verbenaceae), *Senna didymobotrya* Fresenius, and *Senna occidentalis* (L.) (both Fabales: Fabaceae) were grown pesticide-free in pots within the Biological Sciences Greenhouse facility at The Ohio State University (OSU) and received at least 12 hr of natural sunlight per day, supplemented by intense 400-W metal-halide wide-spectrum grow lights. Plant strains originated from the same malarious locality as the mosquito colony, Mbita Point, Nyanza, Kenya. Only *S. didymobotrya* is reported to be indigenous to tropical Africa; the others are invasive alien species from the Americas (see Weber 2003, www.cabi.org/isc/datasheet, www.lucidcentral.org/keys). All grow wild or as ornamentals in North America and are now widespread globally in tropical and subtropical zones. These species were selected on the basis of their high olfactory-induced attraction rankings (Nikbakhtzadeh et al. 2014).

Mosquito Production

The Mbita strain of *Anopheles gambiae* Giles used in this study was derived from a colony established and identified by staff at the Odhiambo Campus of the International Centre of Insect Physiology & Ecology at Mbita Point (00° 26'–27' S, 34° 12'–13' E), Suba

District, Nyanza, Kenya, in 2001. The founding specimens were collected in the surrounding community. Our sub-colony at OSU was maintained in accordance with human subjects and biohazards regulations (Institutional Review Board permit 2004H0193, Institutional Biosafety Committee permit 2005R0020). Females were blood-fed for 15 min from a human arm twice per week. Adults were maintained in small acrylic cages (26.7 by 20.3 by 14 cm) in the OSU mosquito insectary (27 ± 1°C, 75 ± 5% RH, photoperiod of 12:12 [L:D] h, with 1.5-h crepuscular periods) and had continuous access to aged tap water and 10% (w/v) sucrose-soaked cotton wicks. Eggs, laid in half-filled water dishes (3.3 cm in height, 9 cm in diameter), hatched 2 d later. Groups of 100 first-instar larvae were transferred to shallow pans (24 by 24 by 6 cm) and fed a fixed regimen of powdered Tetramin fish food (Tropical Flakes, Tetra Holding, Inc., Blacksburg, VA) until pupation (Gary and Foster 2001). Adults used in experiments had wing lengths (mean ± SE, n = 30, each sex) of 3.12 ± 0.014 mm (males) and 3.31 ± 0.016 mm (females).

Experimental Cages

Six clear acrylic plastic test cages (66 by 50 by 67 cm, length by width by height) were placed in a climate-controlled greenhouse room (3.6 by 2.7 m; ambient means outside the cages: 27.7°C, 31.6% RH). Four 400-W metal-halide growing lights were suspended 120 cm above the cages. Each cage had one sleeved access opening (15 cm in diameter) and one screened window (12 cm in diameter) on its front side. Cages sat on concrete blocks covered by white plastic sheets, approximately 20 cm above the floor, immersed in trays of soapy water, creating moats to prevent entry of any cockroaches and ants not already eliminated from the room by pit traps and nonvolatile toxic baits.

Mosquitoes in each cage had access to water and a black container on its side that served as a daytime resting site. Each of the four species of plants was placed in a separate cage, and two cages were used for positive and negative controls: 10% sucrose solution and water only, respectively. The positive control had four sucrose-soaked and two water-soaked cotton wicks inserted in vials containing the liquids, while the negative control had four water-soaked cotton wicks. The plant cages had two water wicks in addition to either one or two of the experimental plants, depending on their size: cages with large plants (*S. didymobotrya* and *S. occidentalis*) had one per cage; cages with small plants (*P. hysterophorus* and *L. camara*) had two. The plants varied significantly in architecture, but their volumes were approximately 20 by 15 by 25 cm for small plants and 40 by 35 by 60 cm for large plants. The soil surface of the pots was completely covered by white nylon fabric and held tightly to the plant's basal stem by plant tape, making dead mosquitoes easily visible on the pot as well as on the cage floor but allowing for daily watering. For the sake of uniformity, each control cage also contained a soil-filled pot, covered in white fabric but lacking a plant, and given 50 ml of water daily. HOBO data loggers (Onset, Bourne, MA) recorded temperature and relative humidity (RH) inside each cage every 30 min during the experiments (Table 1).

Experimental Procedures

Survival

In each of five replicate experiments, after overnight emergence, 14 mosquitoes of each sex (n = 28) were transferred by aspirator to 400-ml plastic holding containers and transported to the greenhouse test room. Just prior to introducing the plants into the experimental cages, they were washed by pressurized water to remove predators

Table 1. Mean $\pm$ SE (based on Kaplan–Meier estimates) and median $\pm$ MAD of survival for *Anopheles gambiae*, males and females combined, exposed to four attractive plants and two control cages

Experimental groups	Mean $\pm$ SE of survival (days)	Median $\pm$ MAD ^a of survival (days)	Mean temp °C, % RH ^b
Water (negative control)	2.94 $\pm$ 0.11a	3.0 $\pm$ 1	21.5, 57.6
<i>Parthenium hysterophorus</i>	5.97 $\pm$ 0.67b	4.0 $\pm$ 1	23.8, 76.8
<i>Lantana camara</i>	10.31 $\pm$ 0.87c	9.0 $\pm$ 6	22.5, 88.6
10% sucrose (positive control)	12.60 $\pm$ 1.16c	9.5 $\pm$ 7	22.6, 57.9
<i>Senna occidentalis</i>	13.69 $\pm$ 1.00c	14.0 $\pm$ 7.5	22.7, 81.1
<i>Senna didymobotrya</i>	19.00 $\pm$ 1.13d	24.0 $\pm$ 3	20.5, 95.1

n = 5 replicates. Mean values followed by same letter are not significantly different.

^a MAD = median absolute deviation.

^b Inside cages, *n* = 200 recordings.

and plant-feeding insects. *Parthenium hysterophorus* and *L. camara*, in small pots, received 50 ml of tap water per day, while the larger pots of *S. didymobotrya* and *S. occidentalis* received 70 ml of water per day. All except *S. didymobotrya* were bearing flowers throughout the experimental period. The 28 mosquitoes were released into each test cage at around 1600 hours. The number of mosquitoes per treatment group per replicate was kept small to minimize the possibility of competition for sugar sources. The release date was designated as day zero (adults <24-hr-old), and mortality was recorded every day for 25 d. Cages were inspected daily at 1600 hours, and dead mosquitoes were collected, sorted by sex, and counted. Live mosquitoes also were counted each day to confirm totals and verify that none had been overlooked or had escaped. Fallen plant materials were wiped off the cage floors with moist paper napkins, as required. Fresh water and sugar solutions, as well as wicks, were replaced every 2 d. This experiment was repeated five times for each plant and the two control groups (*n* = 5). Daily mortality data were used to create a life table for each cage. Life tables were arranged for both pooled and sex-segregated data.

Fructose Content

Twenty 1-d-old mosquitoes (sex ratio 1:1) were likewise placed at 1600 hours in each of six cages, providing the same six treatment groups as above. Mosquitoes remained in the cages until just before sunrise (approximately for 13 hr), when all were removed. Collected mosquitoes were immediately transferred to a -60°C freezer in containers with screened lids, to halt digestion. Specimens were tested later the same day for the presence of fructose in the crop, using the cold anthrone method of Van Handel (1972), as modified for estimates of quantity (Haramis and Foster 1983). Experiments were repeated four times (*n* = 4) for each plant group and the controls.

Data Analysis

The JMP statistical package (Ver. 10, SAS Institute 2012) was used in all analyses. Figures 2 and 3 were prepared in Microsoft Excel (ver. 2007). Survival curves were constructed with the Kaplan–Meier estimator and analyzed by Wilcoxon tests. This was followed by a proportional-hazards test to determine if there were significant differences in risk ratio between treatments. The proportions of mosquitoes with any fructose (fructose positivity) were analyzed using likelihood-ratio, Pearson's chi-square, and Fisher's exact tests.

A Shapiro–Wilk test for normality of the data on fructose quantity among the same samples, including those mosquitoes that were fructose-negative, was followed by analysis with a Kruskal–Wallis ANOVA and Wilcoxon pairwise tests, to detect significant treatment-group differences.

Results

Survival Rate

Both average mean and average median survival rankings, according to treatment, were as follows: *S. didymobotrya* > *S. occidentalis* > 10% sucrose > *L. camara* > *P. hysterophorus* > water (Table 1). Analysis of survival curves by Wilcoxon test revealed a significant difference by plant treatment ($P < 0.0001$), but not by sex ($P = 0.20$). Likewise, there were no significant differences between sexes within each plant treatment (*P. hysterophorus*, $P = 0.86$; *S. didymobotrya*, $P = 0.15$; *S. occidentalis*, $P = 0.47$; *L. camara*, $P = 0.06$; Sugar, $P = 0.39$; Water, $P = 0.18$). Therefore, data from the sexes were combined for pairwise comparison of survival curves.

The water-only group had significantly lower survivorship than all other treatments ($P \leq 0.0001$; Bonferroni-corrected alpha = 0.0033, here and below). Among the other treatments, mosquitoes exposed to *S. didymobotrya* survived significantly longer than the other four (cf. *S. occidentalis*, $P = 0.0015$; *L. camara*, $P < 0.0001$; *P. hysterophorus*, $P < 0.0001$; 10% sucrose, $P = 0.0003$; Fig. 1; Table 1). Mosquitoes exposed to *L. camara*, *S. occidentalis*, or 10% sucrose were not significantly different from each other ($P = 0.02$, $P = 0.35$, and $P = 0.28$, respectively) and occupied a middle category, extending life longer than both water and *P. hysterophorus*, but shorter than *S. didymobotrya*. Despite *P. hysterophorus*' much lower survival value than *S. occidentalis*, *L. camara*, or 10% sucrose ($P < 0.0001$, $P = 0.0003$, and $P < 0.0001$ respectively), it prolonged life significantly longer than water alone ($P = 0.0001$), and a few individuals survived almost as long as those on *L. camara*, up to 25 d.

A proportional-hazards test, which allowed for the inclusion of differences in the survival of sexes, was used to calculate risk ratios. When treatment and sex were included in the model, both had significant effects according to a likelihood-ratio test ($P < 0.0001$ and $P = 0.02$). However, when the interaction between the two was included, it was found not to have a significant impact ($P = 0.42$). Therefore, in the chi-square comparisons of risk ratios, only sex and treatment were used (Bonferroni-corrected alpha = 0.0033). Compared with the water treatments, all other treatments had significantly lower risk ratios, and thus higher survival ($P < 0.0001$ for all). *Senna didymobotrya* had significantly lower risk ratios than the other three plants and also lower than 10% sucrose (cf. *S. occidentalis*, $P = 0.0014$; cf. *L. camara*, $P < 0.0001$; cf. *P. hysterophorus*, $P < 0.0001$; and cf. sucrose, $P = 0.0002$), whereas *P. hysterophorus* gave significantly higher risk ratios (cf. *S. occidentalis*, $P < 0.0001$; cf. *L. camara*, $P = 0.0005$; cf. 10% sucrose, $P < 0.0001$). Finally, *L. camara* and *S. occidentalis* did not have significantly different risk ratios from either each other or from 10% sucrose ($P = 0.01$, $P = 0.04$, and $P = 0.60$, respectively). While all mosquitoes with only water died in fewer than 5 d, at the other extreme, half of those exposed to *S. didymobotrya* were still alive on day 25 (Fig. 1). Mosquitoes on *P. hysterophorus* showed a sharp early drop in survival, approximately 70% of them dying within the first 5 d, but a few survivors lived considerably longer (Fig. 1). The populations of mosquitoes on 10% sucrose, *S. occidentalis*, and *L. camara* gradually decreased, and only 21, 18, and 8%, respectively, were alive on day 25 (Fig. 1a, pooled data).

Fig. 1. Survival curves of (a) pooled, (b) males, and (c) females, according to exposure to four different plant species (SD = *S. didymobotrya*, and SO = *S. occidentalis*) and two controls: negative (water) and positive (10% sucrose; $n=5$). Survival curves were constructed using Kaplan–Meier estimators and compared by Wilcoxon tests. This was followed by a proportional hazards test.

Fructose Content

Cold anthrone tests demonstrated differences in the presence and amount of fructose among mosquitoes exposed to different species of plants. The ranking of mean proportions positive for fructose according to treatment, both sexes combined, was as follows: 10% sucrose > *L. camara* > *S. occidentalis* > *S. didymobotrya* > *P.*

Fig. 2. Proportion of fructose-positive *Anopheles gambiae* (pooled males and females). Twenty mosquitoes in each replicate. Sugar intake rate was analyzed with likelihood ratio, Pearson's chi-square, and Fisher's exact tests. Error bars were estimated using an Agresti–Coull interval. Values headed by the same letters are not significant by a Bonferroni adjustment for multiple comparisons between values ($\alpha=0.0033$). For individual pairwise comparisons, see text.

hystrophorus > water (Fig. 2). To determine if the proportion of mosquitoes with any detectable fructose varied significantly by treatment, we first tested for differences between replicates using likelihood ratio and chi-square, and no significant differences were found ($P=0.93$ and $P=0.93$). Differences according to sex also were insignificant ($P=0.73$ and $P=0.73$). Therefore, data on replicates and sexes were combined, and significant differences among treatments were detected by these two tests ($P<0.0001$ for both; Fig. 2). Pairwise comparisons between treatments were made with Fisher's exact tests, which showed the following differences: 10% sucrose versus all other treatments ($P<0.0001$); only water versus *L. camara*, *S. didymobotrya*, and *S. occidentalis* ($P=0.003$, $P=0.04$, and $P=0.01$, respectively); *S. occidentalis* versus *P. hystrophorus* ($P=0.028$); and *L. camara* versus *P. hystrophorus* ($P=0.01$). However, with application of the Bonferroni-corrected alpha value of 0.0033 for multiple comparisons, only 10% sucrose versus all other treatments and water only versus *L. camara* were significantly different in fructose positivity.

The ranking of mean quantities of fructose according to treatment, both sexes combined, was as follows: 10% sucrose > *S. occidentalis* > *S. didymobotrya* > *L. camara* > *P. hystrophorus* > water (Fig. 3). The distribution of amounts was skewed (Shapiro–Wilk goodness-of-fit, $P<0.001$), so nonparametric methods were applied, to detect significant differences in amounts of sugar among fructose-positive mosquitoes. No significant effect of sex on amount of fructose was detected (Wilcoxon test, $P=0.74$). Therefore, data from each sex were combined for the following tests. Differences in amount, according to treatment, were significant (Kruskal–Wallis test, $P<0.0001$). Pairwise comparisons of treatments by Wilcoxon tests showed the following differences: 10% sucrose versus all other treatments ($P<0.0001$); water versus *L. camara*, *S. didymobotrya*, and *S. occidentalis* ($P=0.0034$, $P=0.02$, and $P=0.008$, respectively); *S. occidentalis* versus *P. hystrophorus* ($P=0.02$); and *L. camara* versus *P. hystrophorus* ($P=0.009$). Comparisons between other treatments were not significant (for each, $P>0.05$). Again, using a Bonferroni-corrected alpha value of 0.0033 for multiple comparisons, only the comparisons between sucrose and other treatments were significantly different in amount of fructose present.

Fig. 3. Mean ($\pm$ SE) fructose quantity (μg) contained in individual mosquitoes, according to treatment group, including fructose-negatives. The distribution of amounts was analyzed for normality by a Shapiro–Wilk test, followed by analysis with Kruskal–Wallis ANOVA and Wilcoxon pairwise tests. Values headed by the same letters are not significant by a Bonferroni adjustment for multiple comparisons between values ($\alpha = 0.0033$). For individual pairwise comparisons, see text.

Discussion

These new results support the contention that female and male *An. gambiae* are particularly attracted to plant species that also prolong their survival by providing abundant sugar. In a cage survival study and a plant-choice study, this generalization was reported by Manda et al. (2007b) for some of the same plant species in the same locality in Kenya. It was further supported by field, olfactometer, and cage studies in Burkina Faso (Gouagna et al. 2010) and Reunion (Gouagna et al. 2014). The apparent connection between attractiveness and endemism, as indicated by our results with *S. didymobotrya*, makes a particularly tempting argument for possible adaptation by the mosquito to this particular plant. The laboratory tests by Nyasembe et al. (2012, 2015) and Nikbakhtzadeh et al. (2014), which included clearly unattractive plants, also noticed this connection, though with inconsistencies. For example, *Ricinus communis* L. provided similar longevity in a cage but was less attractive in a no-choice olfactometer than *P. hysterothorus* (Nyasembe et al. 2012, 2015). The null hypothesis might be that visual or olfactory cues used by nonpollinating mosquitoes to locate plant hosts are randomly associated with various plant species without regard to sugar. This is a reasonable assumption, because many of the test species in these studies are exotic to Africa, perhaps allowing insufficient time for adaptive local-plant preferences of *An. gambiae* to evolve. Yet, the results of Manda et al. (2007a,b), Stone et al. (2012), B. Ebrahimi (unpublished data), and the present study all suggest that an attraction–sugar association does exist. The outlying exception is *P. hysterothorus*.

In the particular case of *P. hysterothorus*, the current study lends support to the conclusion by Manda et al. (2007b) that this plant is an exception to the attraction–value rule, being highly attractive according to their multiple-choice cage assay, yet having little survival value. One dissonant feature of that study is that in a companion publication, they reported fairly high fructose-positive rates in mosquitoes housed with that plant (Manda et al. 2007a). The studies of Nyasembe et al. (2012, 2015), on the other hand, found not only that *P. hysterothorus* is highly attractive in an olfactometer but also that it both provides abundant sugar and promotes long life in cages, a direct contradiction to our results and tentative conclusions. In the

Nyasembe et al. investigation, the plants were acquired from the Nairobi area of central Kenya, and the mosquito strain came from the lower-altitude Mbita Point location in western Kenya. Nyasembe et al. (2015) have suggested that plant biotypes differing in nectar production in different localities may explain these conflicting results. This idea would presuppose that *P. hysterothorus*, despite having invaded Africa from South America only a little over a century ago has established a patchwork of populations, some of which, in the absence of its ancestral pollinators, no longer provide much nectar, while both high-nectar and low-nectar populations maintain a universally attractive blend of volatiles for its pollinators.

An additional view of this inconsistency is suggested by one feature of our survival data on *P. hysterothorus*: A few mosquitoes in each of the treatment groups lived much longer (15–25 d) than would be expected from the short median time of death (4 d), which was not much better than water (3 d). This prolonged survival among a few individuals suggests either that the plant became a limited but reliable sugar source when competition diminished, after the majority of mosquitoes had died, or that those few survivors were able to obtain sugar by chance or an inherent or experience-acquired skill at locating the sugar. It also should be noted that even though this plant species appears to be a poor source of sugar in at least one locality where malaria transmission is common, mosquitoes may gain other benefits, because *P. hysterothorus* contains the toxin parthenin (Nyasembe et al. 2015), which appears to inhibit malaria parasite development within the mosquito (Manda 2007, J.N. Balaich, unpublished data). It will be important to resolve the puzzle of the value of *P. hysterothorus* to *An. gambiae* to understand its possible impact on malaria transmission (Nyasembe et al. 2015).

The sugar-content data on *L. camara* might appear to be another mosquito–plant anomaly. Although this plant ranked first in the proportion of mosquitoes obtaining at least some sugar, it ranked third in amounts of sugar ingested, and that latter ranking corresponded to its survival value and its attractiveness (Nikbakhtzadeh et al. 2014). This suggests that *L. camara*’s nectaries are abundant or easily accessed, yet provide sugar in limited quantities. Our tentative hypothesis is that sugar is taken primarily from inconspicuous glands around the calyx of the flower head. *Anopheles gambiae* s.l. readily inserts its proboscis into the florets’ corollas, in the field as well as in cages, but without sustained insertion or obvious ingestion of nectar (W.A.F., unpublished data). But persistent probing occurs particularly among the interstices of the calyces. These observations suggest that the mosquitoes generally obtain scant floral nectar but also can pick up small amounts of extrafloral nectar in the calyx where muscoid flies and other diurnal insects also commonly probe and where pipette rinses are positive for fructose (W.A.F., unpublished data). On the other hand, in the laboratory, we have observed that the two *Senna* species produce abundant nectar from their extrafloral nectaries, which explains the higher fructose contents of mosquitoes housed with them. We have never seen mosquitoes probing on their typical legume-structured flowers or gaining access to their floral nectaries in the laboratory or field. Thus, the presence or absence of flowers on our experimental *Senna* plants was probably inconsequential. This does not hold true for *P. hysterothorus*, on which we have observed that the flowers are probed preferentially and are the likely source of nectar.

The curious observation that *S. didymobotrya* prolonged mosquito life significantly more than the 10% sucrose treatment bears further examination. The cause may be simple: the plant could have offered more concentrated or easily found sources of sugar than the

four sucrose wicks. We have observed that both the bracts on the meristems of this species and at the edges of its numerous leaflets can produce extrafloral nectar (C.M. Stone, B.T. Jackson, B. Ebrahimi, and W.A.F., unpublished data). Alternatively, the plant may have provided a more amenable microclimate, e.g., the relative humidity recorded was substantially higher, so damaging flight activity in search of water would be infrequent. The most easily conceivable chemical explanation would be the presence of amino acids in the nectar, a common occurrence among plants (Baker and Baker 1973), which can prolong life in mosquitoes (Eischen and Foster 1983, Vrzal et al. 2010).

Our current attempt to differentiate among plant species according to their ability to provide sugar and prolong life requires some caveats. One is that the number of plant species tested was small. Yet, the results corroborate those of Manda et al. (2007a,b), whose cage study included many more species and whose rankings were similar. In both studies, the ranks are only suggestive when the differences discussed are not statistically significant. A second caveat regards mosquito fructose contents. The data are not accurate measures of the amount ingested during the night, because there were undoubtedly differences in concentration, composition, digestion rate, and the time elapsed since feeding occurred. But about half of the sugar in floral and extrafloral nectars consists of the monosaccharide fructose or the fructose moiety of the fructose-glucose disaccharide sucrose (Nicolson and Thornburg 2007), and for all treatment groups, we halted digestion soon after the nighttime feeding period. Thus, the measures roughly indicate relative sugar availability on the plants. A third caveat is that the experimental plant species were not strictly comparable, because they inevitably differed in total bulk and in the surface areas of flowers, leaves, and stems. If they had been comparable in terms of presence and numbers of flowers, extrafloral nectaries, and tissues capable of being pierced (an undocumented possibility for *An. gambiae*), their relative intrinsic value as sugar sources inferred from this experimental setup might have been more realistic. However, even by that high standard, their utility for natural mosquito populations likely would be obscured by their differences in size and abundance in the field. That, ultimately, is where the nutritional contribution of a plant species to *Anopheles* is determined.

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