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## Attraction of *Stomoxys* sp. to various fruits and flowers in Mali

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## Attraction of *Stomoxys* sp. to various fruits and flowers in Mali

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**Abstract.** The attraction of three *Stomoxys* species to 26 fruits and 26 flowers of different plant species was investigated in two different sites in Mali during 2008. *Stomoxys niger bilineatus* Grunberg (Diptera: Muscidae) was attracted to a wider spectrum of species, significantly attracted by four fruits and eight flowers compared with control traps, whereas *S. sitiens* Rondani (Diptera: Muscidae) was attracted to six fruits and seven flowers of different plants, and *S. calcitrans* L. (Diptera: Muscidae) was only attracted to one fruit and three flowers. Cold anthrone assays showed a significantly higher prevalence of sugar feeding amongst all three species at the lagoon site than at the site near Mopti. The rhythm of activity study shows temporally separated blood- and sugar-feeding periods for *S. niger bilineatus* and *S. sitiens*, but not for *S. calcitrans*. A comparison between blood and sugar feeding throughout the day shows that sugar feeding activity is as frequent as blood feeding activity. Because not much is known about the preferred sugar sources for *Stomoxys* species in their natural habitats, the present study provides valuable information regarding the attraction capability of several plants with possible future implication for *Stomoxys* control strategies.

**Key words.** *Stomoxys calcitrans*, *S. niger bilineatus*, *Stomoxys sitiens*, glue traps, nectar feeding, stable flies.

### Introduction

Biting stable flies of the genus *Stomoxys* are considered major pests of pasture cattle and livestock (Hogsette & Farkas, 2000; Hogsette *et al.*, 2008). Both male and female adults require three to five bloodmeals for successful mating, inflicting painful bites and subsequent bleeding which can result in lower average weight and decreased milk production, with enormous economical losses worldwide (Bruce & Decker, 1958; Campbell *et al.*, 1987, 2001). In

the US alone losses to the cattle industry are estimated at over 1 billion \$US annually (Taylor & Berkebile, 2006). Furthermore, *Stomoxys* is known to obtain bloodmeals from human sources when cattle or other animal hosts are lacking, and can be a major nuisance having major adverse effects on the tourism industry in areas prone to *Stomoxys* infestations (Hogsette *et al.*, 1987). Zumpt (1973) lists the 18 known species of *Stomoxys*, with 14 of these found on the African continent (Mihok *et al.*, 1995a). *Stomoxys* has been shown to transmit several

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parasitic organisms mechanically, such as *Anaplasma marginale* (Theiler) (Potgieter *et al.*, 1981; Scoles *et al.*, 2005), *Trypanosoma evansi* (D'Amico *et al.*, 1996; Sumba *et al.*, 1998), *T. brucei* and *T. vivax* (Mihok *et al.*, 1995b), *T. congolense* (Sumba *et al.*, 1998), capripox virus and African swine fever virus (Mellor *et al.*, 1987). However, the few field transmission studies have shown *Stomoxys* to have a limited potential as vectors.

Although bloodmeals are the main protein source of the stable flies, and are a mandatory prerequisite for successful mating and ovarian development, observations of stable flies on flowering plants in the field (Eavy *et al.*, 1981; Tseng *et al.*, 1983) have suggested *Stomoxys* species may be imbibing nectar to supplement their bloodmeal diet. Indeed, a significant portion of *Stomoxys* flies have been shown to feed on plant-derived sugars (Mihok & Clausen, 1996; Taylor & Berkebile, 2008), especially when hosts which provide bloodmeals are scarce (Jones *et al.*, 1985). *Stomoxys* flies are known to consume sugars within a few hours of eclosion, and were shown to continue feeding on sugars even when blood was supplied *ad libitum* (Jones *et al.*, 1985). Sucrose solutions as well as flower cuttings and pollen extracts of selected wild plants have prolonged *Stomoxys* survival compared with water controls (Tseng *et al.*, 1983; Jones *et al.*, 1985), although not as much as bloodmeals (Moobola & Cupp, 1978). Anthrone assays of wild *Stomoxys* populations in rural and urban locations in Nebraska revealed a larger per cent of sugar-fed stable flies compared with blood-fed, with a small correlation between nectar and blood feeding. This suggests that sugar feeding may increase the chance for successful blood-feeding by the stable flies (Taylor & Berkebile, 2008).

Sugar feeding seems to be an important energy source for the stable fly (Hogsette *et al.*, 1987), yet little is known about the preferences and attraction signals which are sensed by *Stomoxys* sp. Tseng *et al.* (1983) found that *Stomoxys* are most attracted to golden rod (*Solidago fistulosa*) and salt bush (*Baccharis halimifolia*) flowers in FL, U.S.A. *Stomoxys* flies contaminated with willow (*Salix* sp.) pollen have also been reported (Tseng *et al.*, 1983; Jarzen & Hogsette, 2008). The attraction of selected volatile compounds was compared in an attempt to increase trap efficiencies, however, the attractants were mainly related to blood-seeking or mating behaviour, and not to sugar-seeking behaviour (Mihok *et al.*, 1995a). To date, very few studies have investigated the relationship between *Stomoxys* sp. and various plant species which exist in its natural environment.

In a previous field experiment which examined the attraction of *Anopheles gambiae* s.s. Giles to various fruit and flowers in Mali, a considerable number of *Stomoxys* flies were trapped along with the mosquitoes. Interestingly, a distinct preference to several fruit and flower baits by three *Stomoxys* species, namely *S. calcitrans* L., *S. niger bilineatus* Grunberg and *S. sitchensis* Rondani, was observed. In the present study, we report the attraction efficiency of selected fruit and flowers to, as well as the diurnal feeding behaviour of, the three aforementioned species.

## Materials and methods

### Trap description

The attractiveness of fruits, seed pods and flowers was determined using a specially designed sticky trap which was constructed as follows: 1.5-L plastic bottles were cut in half and the bottom halves buried in the ground so the rims of the bottles were slightly higher than the soil surface. The bottles were then filled with water. Soil around the bottles was moistened with water and packed down to stabilize the bottles.

Stiff (0.2-cm thick) plastic mesh, 70 × 70 cm, with 0.8-cm square holes, was rolled into cylinders and secured with plastic tie wraps. The tops were closed with circular pieces of the same material, also secured with plastic tie wraps. Each mesh cylinder was placed above a buried bottle. Before the mesh cylinders were secured in place, one of the following was placed inside: 0.5 kg of ripe fruit pieces arranged on wooden stakes (40 cm long), or 0.5 kg of freshly cut flowers (50–70 cm long) placed in the bottle of water. The cylinders were then fixed to the ground with 20-cm-long wooden stakes and painted with an adhesive (Tanglefoot; Tel Aviv, Israel) to capture insects that were attracted to these baits.

### Site description

The Internal Delta of the Niger River, the main delta and associated wetlands, comprise an area of about 30 000 km<sup>2</sup> at an altitude of 263–271 m a.s.l. The delta proper is located in the centre of Mali and is about 400 km long, oriented SW-NE, with a mean width of about 90 km. There is a population density of 500/km<sup>2</sup> in the Delta, compared with a population density of 9/km<sup>2</sup> in the remainder of the country. Recently an agro-pastoral system became established in which resident farmers grow rice and millet and at the same time produce their own cattle. Every year for 7–8 months, the pastures of the delta are grazed by 1 200 000 cattle and 1 500 000 sheep and goats, including the livestock of the semi-nomadic populations living nearby. Direct precipitation over the delta varies by year and location, with annual totals ranging from 200 to 600 mm. The rainy season usually extends from July through to mid-October in the south, and from mid-July to mid-September in the north.

Some experiments were conducted at a natural site near a seasonal lagoon (15°0'0"N, 4°10'0"W) approximately 50 km north of Sevare. The lagoon was about 4 km long and surrounded by wooded grassland dominated by *Acacia* species. The site was visited daily by several groups of semi-nomads with about 500–1000 cattle. Every day, villagers working in nearby rice fields grazed several dozen tethered donkeys near the lagoon. Experiments were also conducted at a site at the outskirts of Mopti (14°30'0"N, 4°12'0"W) in the middle of rice fields, about 10 km southwest of Sevare. Every day, donkeys and goats were grazing along the roads and trails crossing the rice fields.

*Stomoxys attraction to fruit*

In November 2008, 28 sticky traps were placed at 20-m intervals along the shore of the seasonal lagoon. In total, 26 different fruit species (Table 1) and two controls were used as baits. Controls consisted of one unbaited sticky trap and one sticky trap baited with a sponge soaked with white-refined sugar solution.

Treatments (fruits and controls) were randomly assigned to four blocks of seven sticky traps, and placed in their assigned sticky traps at night. After 24 hours, *Stomoxys* spp. were removed from the sticky traps, counted and stored in 70% ethanol for later identification to species in the laboratory. Treatments were then rotated to the next block, and the adhesive was reapplied to the cleaned sticky traps, which were placed over the treatments for another 24 h. This was repeated for seven consecutive nights.

*Stomoxys attraction to flowers*

In late October 2008, 28 sticky traps were placed at 20-m intervals along a road between rice fields at the Mopti site. Flowers of 26 different plant species (Table 2), collected within a 20-km radius of the Mopti site, and two controls were used as baits. Controls consisted of one unbaited sticky trap and one sticky trap baited with a sponge soaked with white-refined sugar solution.

Treatments (flowers and controls) were randomly assigned to four blocks of seven sticky traps, and placed in their assigned sticky traps at night. After 24 h, *Stomoxys* spp. were removed from the sticky traps, counted and stored in 70% ethanol for later identification to species in the laboratory. Treatments were then rotated to the next block, and the adhesive was reapplied to the cleaned sticky traps, which were placed over the treatments for another 24 h. This was repeated for six consecutive nights.

*Stomoxys sugar feeding*

To assess the percentage of flies which fed on sugar before blood-feeding, cold anthrone assays as described by Van Handel (1967) were performed on three groups of 100 flies of each species, collected randomly with a sweep net in the vicinity of a donkey at the lagoon and at the Mopti sites. Flies which showed obvious signs of recent blood feeding were discarded, but the remainder were tested on site.

*Rhythm of activity*

Diurnal feeding behaviour of the three *Stomoxys* species was investigated at the seasonal lagoon described above during mid-November 2008. Flies were collected from two traps baited with flowering *Acacia albida*, and from two donkeys using sweep nets. Collections were performed during 30-min intervals from 05.30 until 21.00.00 hours for three consecutive days and replicated six times for each interval: two donkeys

or two groups of plant-baited sticky traps (six traps per group, data pooled)  $\times$  3 days = 6 reps.

*Weather*

The weather in Mali during October and November is very constant, with average temperatures ranging from 30 and 20 °C, and <1 rain day per month (<http://www.weatherforecast.com/mali/mopti/>). Thus, changes in weather did not interfere with our tests and weather variables were not recorded.

*Statistical analysis*

Fruit and flower data were first normalized by conversion to  $\log_{10}(n + 1)$  then subjected to general linear models (GLM) (SAS Institute, 2003) using the following main effects model statements: Stable flies captured = Treatment Block Day, where treatment was either fruits or flowers, block was a grouping of seven treatments and day was one of the consecutive trapping days of each study. Relative attraction ratios were calculated for the fruit and flower data by dividing the mean number of flies captured by each treatment by the mean number of flies captured by their respective control traps.

Cold anthrone assay data were normalized by conversion to  $\log_{10}(n + 1)$  then subjected to GLM (SAS Institute, 2003) using the following main effects model statements: Per cent sugar fed = Location Species Day, where location was either Mopti or the lagoon sites, species was one of the three *Stomoxys* species and day was one of the consecutive trapping days of each study.

Sugar- and blood-feeding activity data were normalized by conversion to  $\log_{10}(n + 1)$  then subjected to GLM (SAS Institute, 2003) using variations of the following main effects model statements: Flies Captured/Trapped = Bait Time Species Rep, where bait was either the flower or the donkey, time was the capture/trapping time, species was one of the three *Stomoxys* species and rep was one of the six replications performed during three consecutive days. In the model variations, individual class variables were substituted as grouping variables, e.g. when analysed by Bait, the model becomes Flies Captured/Trapped = Time Species Day, and so on.

Means for all analyses above were separated with the Ryan–Einot–Gabriel–Welsch Multiple Range Test (REGWQ), and unless otherwise stated,  $P < 0.05$  (SAS Institute, 2003). Although  $\log_{10}(n + 1)$  values were used for the analyses, actual values are reported in the text, figures and tables. Means reported in the text are in the form  $\bar{x} \pm \text{S.E.}$

**Results**

There were no significant differences between the mean numbers of any *Stomoxys* species captured by the two control traps (empty and sugar water baited), so only the results for the empty control trap are shown. The attraction to fruits and flowers decreased rapidly through the various treatments, so

**Table 1.** Fruits, seed pods or stems from the following 26 plants were evaluated for attraction to *Stomoxys calcitrans*, *S. niger bilineatus* and *S. sitiens* in Mali.

Common name	Family	Plant species
Purchased from the market in Sevaré:		
Date	Arecaceae	<i>Phœnix dactylifera</i> L.
Papaya	Caricaceae	<i>Carica papaya</i> L.
Honey melon	Cucurbitaceae	<i>Cucumis melo</i> L.
Dwarf banana	Musaceae	<i>Musa acuminata</i> Colla
Guava	Myrtaceae	<i>Psidium guajava</i> L.
Sugar cane	Poaceae	<i>Saccharum officinarum</i> L.
From southern Mali or imported:		
Pineapple	Bromeliaceae	<i>Ananas comosus</i> (L.) Merr.
Water melon	Cucurbitaceae	<i>Citrullus lanatus</i> (Thunb.) Matsum. & Nakai
Plantain	Musaceae	<i>Musa × paradisiaca</i> L.
Apple	Rosaceae	<i>Malus domestica</i> Borkh.
Orange	Rutaceae	<i>Citrus sinensis</i> (L.) Osbeck
Bitter orange	Rutaceae	<i>Citrus aurantium</i> L.
Edible fruits and seed pods collected from wild plants and trees in the delta:		
Bush melon	Cucurbitaceae	<i>Citrullus colocynthis</i> (L.) Schrad.
	Ebenaceae	<i>Diospyros mespiliformis</i> Hochst. ex A. DC.
Tamarind	Fabaceae	<i>Tamarindus indica</i> L.
	Fabaceae	<i>Piliostigma reticulatum</i> (DC.) Hochst.
	Fabaceae	<i>Acacia albida</i> Delile
African fig	Moraceae	<i>Ficus thonningii</i> Blume
Jujube	Rhamnaceae	<i>Ziziphus mauritiana</i> Lam.
	Tiliaceae	<i>Grewia bicolor</i> Juss.
Soapberry tree	Zygophyllaceae	<i>Balanites aegyptiacus</i> (L.) Delile
Seed pods used only for animal fodder:		
	Fabaceae	<i>Acacia sieberiana</i> DC.
	Fabaceae	<i>Acacia nilotica</i> (L.) Delile
	Fabaceae	<i>Acacia seyal</i> Delile
	Fabaceae	<i>Acacia macrostachya</i> Rchb. ex DC.
Fruits of:		
Kangaroo apple	Solanaceae	<i>Solanum vescum</i> F. Muell.

only the top eight fruits and nine flowers are shown in the tables. Significantly ( $F = 27.86$ , d.f. = 36, 1070,  $P < 0.0001$ ) more flies were captured on flowers ( $2.6 \pm 0.04$ ) than on fruit ( $2.3 \pm 0.03$ ), species ignored.

#### *Stomoxys* attraction to fruit

The main effects model was significant ( $F = 15.14$ , d.f. = 34, 566,  $P < 0.0001$ ) for fly species and fruits, but not for blocks or days. Overall, significantly more *S. niger bilineatus* ( $3.8 \pm 0.06$ ) were attracted to the fruits than *S. calcitrans* ( $1.8 \pm 0.05$ ) and *S. sitiens* ( $1.7 \pm 0.04$ ). Differences in attraction between the latter two species were not significant. The following seven fruits attracted significantly more *Stomoxys*, species overlooked, than the unbaited control trap: *Piliostigma reticulatum*, *Psidium guajava*, *Tamarindus indica*, *Ficus thonningii*, *Saccharum officinarum*, *Cucumis melo* and *Carica papaya*. The overlapping significance levels have been omitted here because they are more meaningful when the fly species are examined individually. There were no significant differences in attraction between the remaining 19 fruits and the unbaited control trap.

The fruit of *Piliostigma reticulatum* was the most attractive to all three *Stomoxys* species and attracted significantly

more individuals than did their respective control traps (Table 3). *Piliostigma reticulatum* attracted significantly more *S. calcitrans* than the other 25 fruits; however, significance levels were wide and overlapping for the other two *Stomoxys* species. The *Stomoxys* species captured in the largest numbers was *S. niger bilineatus*, but the *Stomoxys* species with the highest relative attraction ratios was *S. sitiens*. It is interesting to note that 7 of the 26 fruits evaluated were the most attractive to all 3 *Stomoxys* species, although not entirely in the same order (Table 3). All 26 fruits attracted numerically more *S. sitiens* than did the control trap. However for *S. calcitrans* and *S. niger bilineatus*, 11 fruits, e.g. *Citrus aurantium*, *Musa paradisiaca*, *Solanum vescum*, *Acacia nilotica* and *Ananas comosus* (Table 1), attracted numerically fewer individuals than did their respective control traps. There were no significant differences between blocks or days. The relative attraction to fruits by *S. sitiens* was much higher than it was for the other two *Stomoxys* species (Table 3).

#### *Stomoxys* attraction to flowers

The main effects model was significant ( $F = 32.29$ , d.f. = 34, 503,  $P < 0.0001$ ) for fly species and fruits, but not for blocks or days. Overall, significantly more *S. sitiens*

**Table 2.** Flowers from the following 26 plants were evaluated for attraction to *Stomoxys calcitrans*, *S. niger bilineatus* and *S. sitiens* in Mali.

Family	Plant species
Apocynaceae	<i>Leptadenia pyrotechnica</i> (Forssk.) Decne.
Asclepiadaceae	<i>Calotropis procera</i> (Ait.) Ait. f.
Capparaceae	<i>Boscia angustifolia</i> A. Rich.
Capparidaceae	<i>Gynandropsis gynandra</i> (L.) Briq.
Combretaceae	<i>Guiera senegalensis</i> J. F.Gmel.
Convolvulaceae	<i>Hypomoea asarifolia</i> (Desr.) Roem. & Schult.
Euphorbiaceae	<i>Ricinus communis</i> L.
Fabaceae	<i>Acacia albida</i> (Delile) A.Chev.*
Fabaceae	<i>Acacia macrostachya</i> Reichenb. ex Benth.*
Fabaceae	<i>Acacia nilotica</i> (L.) Willd. ex Delile*
Fabaceae	<i>Acacia senegal</i> Willd.
Fabaceae	<i>Crotalaria retusa</i> L.
Fabaceae	<i>Cassia tora</i> L.
Fabaceae	<i>Cassia occidentalis</i> L.
Fabaceae	<i>Cassia siamea</i> Lam.
Fabaceae	<i>Indigofera astragalina</i> DC.
Fabaceae	<i>Parkinsonia acculeata</i> L.
Lamiaceae	<i>Hyptis suaveolens</i> (L.) Poit.
Malvaceae	<i>Abutilon</i> sp.
Malvaceae	<i>Hybiscus sabdarrifa</i> L.
Pedaliaceae	<i>Rogeria adenophylla</i> J. Gay ex Delile
Pedaliaceae	<i>Sesamum indicum</i> L.
Rhamnaceae	<i>Ziziphus mauritiana</i> Lam.*
Rubiaceae	<i>Mitracarpus scaber</i> Zucc.
Scrophulariaceae	<i>Striga hermontheca</i> (Del.) Benth.
Solanaceae	<i>Physalis</i> sp. (possibly <i>angulata</i> L.)

\*Seed pods from these trees were also tested for *Stomoxys* attraction.

( $4.5 \pm 0.08$ ) were attracted to the flowers than *S. niger bilineatus* ( $2.1 \pm 0.07$ ) and *S. calcitrans* ( $1.8 \pm 0.06$ ). Differences in attraction between the last two species were not significant. Flowers from the following nine plants attracted significantly more *Stomoxys*, species overlooked, than the unbaited control trap: *Acacia albida*, *A. macrostachya*, *Ziziphus mauritiana*, *Guiera senegalensis*, *Boscia angustifolia*, *Leptadenia pyrotechnica*, *A. nilotica*, *A. sieberiana* and *Cassia siamea*. The overlapping significance levels have been omitted here because they are more meaningful when the fly species are examined individually. There were no significant differences in attraction between the flowers of the remaining 17 plants and the unbaited control trap.

The flowers of *A. albida* were the most attractive to *S. calcitrans* and *S. niger bilineatus*, but the flowers of *Acacia macrostachya* were most attractive to *S. sitiens*. These flowers attracted significantly more individuals than did their respective control traps (Table 4). However, the significance levels were wide and overlapping for all three *Stomoxys* species and little can be said about significant differences in attraction. The species captured in the largest numbers was *S. sitiens*, but the highest relative attraction ratios were those of *S. niger bilineatus*. Of the 26 flowers tested, 9 were the most attractive to all three *Stomoxys* species, although not entirely in the same order (Table 4). The unbaited control traps captured numerically more individuals of all three *Stomoxys* species than some of the flower-baited traps. Flowers from 13 plant species, e.g. *Hybiscus sabdarifa*, *Cassia occidentalis*, *Hypomoea asarifolia*

and *Hyptis suaveolens* (Table 2), attracted numerically fewer *S. sitiens* than the unbaited control. There were no significant differences between blocks or days. The relative attraction to flowers by *S. niger bilineatus* was much higher than it was for the other two *Stomoxys* species (Table 4).

#### *Stomoxys sugar feeding*

The percentages of sugar-fed *Stomoxys* of all three species found at the lagoon site were significantly greater than those at the Mopti site (Fig. 1); however, there were no significant differences between the percentages of sugar-fed *Stomoxys* species at either site.

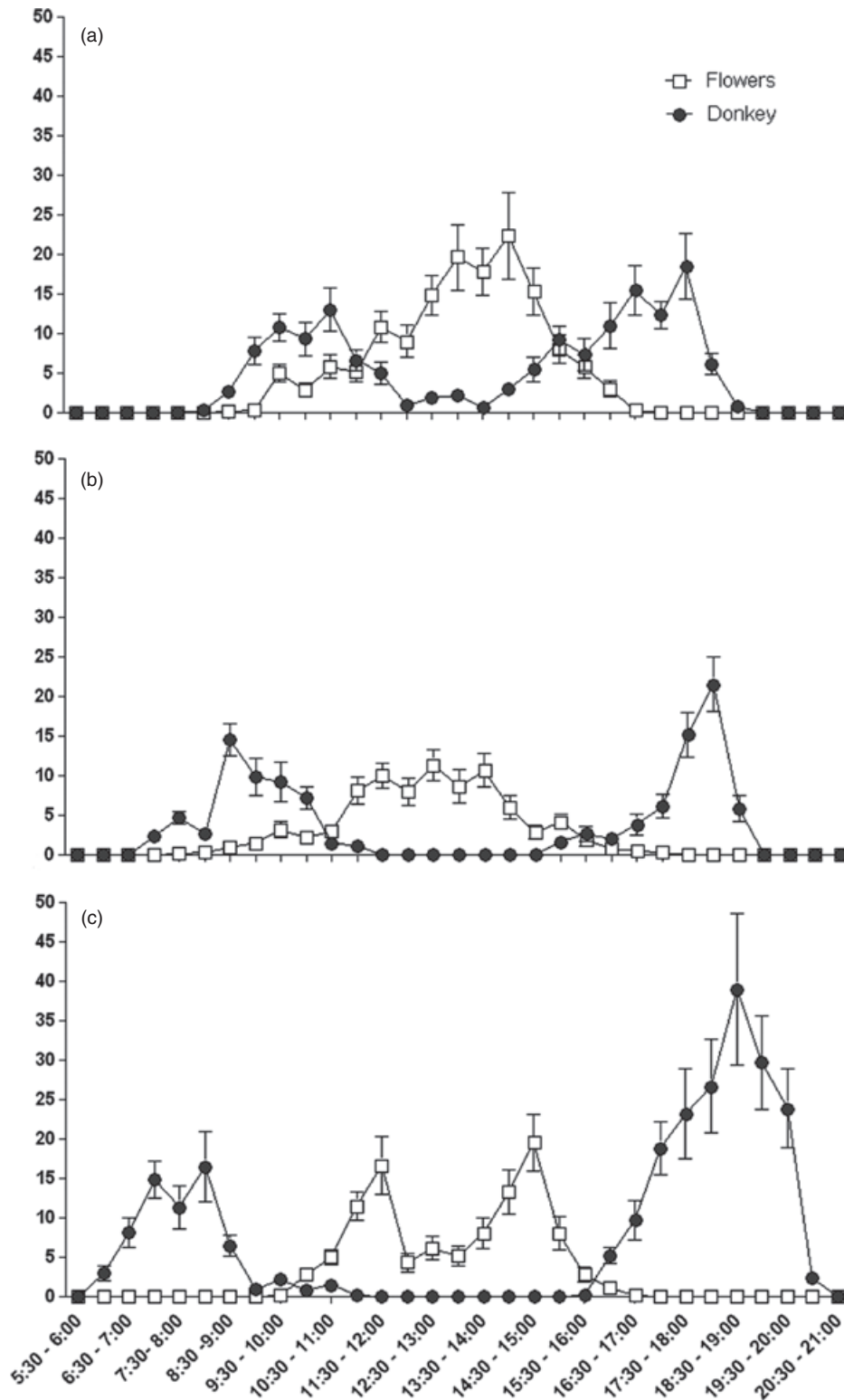
The species with the highest percentage of sugar-fed individuals at both sites was *S. sitiens* ( $33.8 \pm 4.2\%$  at Mopti and  $72.1 \pm 4.1\%$  at the lagoon). The percentage of sugar-fed *S. niger bilineatus* at Mopti was greater than that of *S. calcitrans* ( $25.8 \pm 3.9$  and  $15.3 \pm 2.3\%$ , respectively), but the reverse was found at the lagoon ( $51.6 \pm 5.3$  and  $57.3 \pm 4.9\%$ , respectively).

#### *Diurnal feeding activity*

When analysed by bait type, both models were significant (*A. albida*:  $F = 10.85$ , d.f. = 26, 255,  $P < 0.0001$ ; donkey:  $F = 5.29$ , d.f. = 35, 317,  $P < 0.0001$ ) for time and fly species, but not for rep. The mean number of *S. calcitrans* ( $7.4 \pm 0.09$ ) attracted to *A. albida* was significantly higher than those of *S. niger bilineatus* ( $6.0 \pm 0.09$ ) and *S. sitiens* ( $4.2 \pm 0.07$ ), which were significantly different from each other. The mean number of *S. niger bilineatus* ( $8.8 \pm 0.10$ ) attracted to the donkey was significantly higher than those of *S. caltrans* ( $5.7 \pm 0.07$ ) and *S. sitiens* ( $5.0 \pm 0.08$ ), which were not significantly different from each other.

All three *Stomoxys* species have a blood-feeding activity period in the morning and again in the evening and one sugar-feeding activity period during midday (Fig. 1). The onset of blood-feeding activity for *S. niger bilineatus* was the earliest of the three species, beginning 30 min before sunrise at 06.30 hours, with an acrophase between 08.00 and 08.30 hours. This was followed by a complete cessation of blood-feeding activity between 11.00 and 11.30 hours. Sugar feeding activity began between 09.30 and 10.00 hours with well-defined peaks between 11.30 and 12.00 hours, and between 14.00 and 14.30 hours. This was followed by complete cessation between 16.00 and 16.30 hours. Blood-feeding activity began again between 15.30 and 16.00 hours, peaked between 18.30 and 19.00 hours, and then ended between 20.30 and 21.00 hours, about 2 h after sunset (Fig. 1). There was no significant difference between the morning blood-feeding activity peak ( $16.5 \pm 4.40$ ) and the midday sugar-feeding activity peak ( $19.5 \pm 3.60$ ), but the afternoon blood-feeding activity peak ( $39.0 \pm 9.60$ ) was significant and about twice as high as the morning or midday activity peaks (Fig. 1).

*Stomoxys sitiens*-feeding activity was similar to *S. niger bilineatus*, with some overlap between blood-feeding and



**Fig. 1.** Diurnal feeding behaviour of the three *Stomoxys* species. Average number ( $\pm$  S.E.) of flies caught on blood source or on *Acacia albida*-baited trap hourly throughout the day. Six repetitions for each time period were obtained with two donkeys and two groups of flower-baited traps for three consecutive days. (A) *S. calcitrans*. (B) *S. sitchensis*. (C) *S. niger bilineatus*. The two arrows indicate the time of sun rise and sun set.  $\square$  blood-feeding activity,  $\bullet$  sugar-feeding activity.



sugar-feeding activity periods, but by small numbers of flies. The first blood-feeding activity period began at sunrise (06.30 hours), peaked between 08.30 and 09.00 hours, and ended between 11.30 and 12.00 hours. Sugar-feeding activity began between 07.30 and 08.00 hours, peaked between 12.30 and 14.00 hours, and was followed by complete cessation between 17.00 and 17.30 hours (Fig. 1). The second blood-feeding activity period started between 15.00 and 15.30 hours, peaked between 18.00 and 18.30 hours, and then subsided between 19.00 and 19.30 hours, about 1 h after sunset (Fig. 1). There were no significant differences between the morning blood-feeding activity peaks ( $14.5 \pm 2.08$ ), the midday sugar-feeding activity peak ( $11.3 \pm 1.91$ ) and the afternoon blood-feeding activity peak ( $21.5 \pm 3.45$ ) (Fig. 1).

There was no separation between the sugar-feeding and blood-feeding activity periods for *S. calcitrans* (Fig. 1). One continuous day-long blood-feeding activity period began between 08.00 and 08.30 hours, about 2 h after sunrise, and ended between 18.30 and 19.00 hours. Blood-feeding activity peaked between 10.30 and 11.00 hours, decreased to its low point between 13.30 and 14.00 hours, and then peaked again between 17.30 and 18.00 hours. Sugar-feeding activity occurred throughout most of the blood-feeding activity period starting between 08.30 and 09.00 hours, peaking between 14.00 and 14.30 hours, and ending between 16.30 and 17.00 hours (Fig. 1). *Stomoxys calcitrans* was the only species for which the sugar-feeding activity peak ( $22.3 \pm 5.4$ ) was numerically higher than the morning and afternoon blood-feeding activity peaks ( $13.0 \pm 2.6$  and  $18.4 \pm 4.2$ , respectively). The daily activity period for *S. calcitrans* was also found to be 1 and 3.5 h shorter than those of *S. sitiens* and *S. niger bilineatus*, respectively.

## Discussion

The present study has clearly shown that certain *Stomoxys* species regularly feed on sugars with high prevalence. Other blood-feeding flies, e.g. mosquitoes (Bowen, 1992), phlebotomine sand flies (Schlein & Warburg, 1986), culicoides

(Stewart & Kline, 1999) and tabanids (Foil & Hogsette, 1994), are known to have a similar affinity for carbohydrate sources, and feed more often on sugars than blood (Foster, 1995). Early observations of stable flies visiting flowers, apparently to imbibe nectar, were made, not in Africa, but in Europe (Surcouf, 1921). It has since been shown that stable flies not only visit flowers but actually do feed on nectar (Tseng *et al.*, 1983; Jones *et al.*, 1985). However, regular, long-term feeding on sugar sources has only been shown with *S. calcitrans* in NE, U.S.A. (Taylor & Berkebile, 2008) and with the three *Stomoxys* species we collected in Mali.

When Goodwin *et al.* (1986) conducted studies in Mali, they collected five species in two genera of Stomoxyinae: *Stomoxys calcitrans*, *S. niger bilineatus*, *S. sitiens*, *Haematobia minuta* Bezzi and *H. thirouxi* Roubard. The fact that we collected the same three *Stomoxys* species indicates that they are well established in the region. If other *Stomoxys* species were present, they were not attracted to the fruits and flowers we evaluated. In the present study, *S. sitiens* was most numerous around flowers and *S. niger bilineatus* was most numerous around fruits. It has been reported that *S. calcitrans* can be quite numerous in areas where cattle are being kept by humans, but less numerous in more natural conditions (Ndegwa & Ogado, 2002). This situation did not exist in Mali.

All three *Stomoxys* species showed some degree of attraction to the unbaited glue traps used to cover the fruits and flowers. The addition of a sponge saturated with a sugar solution did not increase this attraction and the addition of some fruits and flowers actually reduced this attraction. The glue traps by themselves probably provided the structure that these flies prefer for resting sites in open areas. This has been observed in agricultural situations where numbers of stable fly adults in open pastures are low unless there are structures, such as feed troughs or mineral boxes, on which the flies can rest (Hogsette *et al.*, 1987). These plastic traps served as passive sticky covers over the various fruits and flowers, and did not demonstrate any degree of attraction that would classify them for use as stand-alone visual traps, such as alsynite (Hogsette & Ruff, 1990) or coroplast (Beresford & Sutcliffe, 2006).

**Table 3.** Mean numbers ( $\pm$  S.E.) and relative attraction ratios of *Stomoxys* species captured at the eight most attractive fruits of selected plant species in Mali ( $n = 6$ ).

Plant species	<i>Stomoxys</i> spp. captured			Relative attraction ratios		
	<i>S. calcitrans</i>	<i>S. niger bilineatus</i>	<i>S. sitiens</i>	<i>S. calcitrans</i>	<i>S. niger bilineatus</i>	<i>S. sitiens</i>
<i>Piliostigma reticulatum</i>	8.1 $\pm$ 1.5a	19.5 $\pm$ 1.2a	4.7 $\pm$ 1.2a	6.1	7.0	10.9
<i>Tamarindus indica</i>	4.8 $\pm$ 1.1ab	10.3 $\pm$ 1.2abcd	3.1 $\pm$ 1.1abcde	3.7	3.7	7.1
<i>Psidium guajava</i>	4.0 $\pm$ 1.2abc	14.9 $\pm$ 1.2ab	4.2 $\pm$ 1.2ab	3.0	5.3	9.8
<i>Cucumis melo</i>	3.5 $\pm$ 1.3abc	7.8 $\pm$ 1.2abcde	2.9 $\pm$ 1.2abcde	2.6	2.8	6.7
<i>Saccharum officinarum</i>	3.3 $\pm$ 1.2abc	6.8 $\pm$ 1.3bcdef	3.8 $\pm$ 1.2abc	2.5	2.4	8.8
<i>Carica papaya</i>	2.6 $\pm$ 1.2abc	5.5 $\pm$ 1.2bcdefg	2.4 $\pm$ 1.1abcdef	2.0	2.0	5.6
<i>Ficus thonningii</i>	2.6 $\pm$ 1.3abc	10.6 $\pm$ 1.1abc	3.4 $\pm$ 1.1abcd	1.9	3.8	7.8
<i>Phoenix dactylifera</i>	2.3 $\pm$ 1.2bc	—	2.4 $\pm$ 1.3abcdef	1.8	—	5.5
<i>Balanitis egyptiacus</i>	—	5.6 $\pm$ 1.2bcdefg	—	—	2.0	—
Control	1.3 $\pm$ 1.3bc	2.8 $\pm$ 1.3efg	0.4 $\pm$ 1.2f	—	—	—

Means in columns followed by the same letter are not significantly different [ $P < 0.05$ ; Ryan–Einot–Gabriel–Welsch Multiple Range Test (SAS Institute, 2003)].

**Table 4.** Mean numbers ( $\pm$  S.E.) and relative attraction ratios of *Stomoxys* species captured at the nine most attractive flowers of selected plant species in Mali ( $n = 6$ ).

Plant species	<i>Stomoxys</i> spp. captured			Relative attraction ratios		
	<i>S. calcitrans</i>	<i>S. niger bilineatus</i>	<i>S. sitiens</i>	<i>S. calcitrans</i>	<i>S. niger bilineatus</i>	<i>S. sitiens</i>
<i>Acacia albida</i>	11.5 $\pm$ 1.2a	15.3 $\pm$ 1.2a	22.2 $\pm$ 1.2a	12.7	21.9	8.5
<i>Ziziphus mauritiana</i>	8.7 $\pm$ 1.2ab	9.7 $\pm$ 1.2ab	18.9 $\pm$ 1.2ab	9.7	13.9	7.3
<i>Acacia macrostachya</i>	7.0 $\pm$ 1.2abc	13.3 $\pm$ 1.1ab	27.3 $\pm$ 1.2ab	7.8	19.0	10.5
<i>Leptadenia pyrotechnica</i>	5.2 $\pm$ 1.2abcd	7.2 $\pm$ 1.2abc	13.8 $\pm$ 1.2abcd	5.8	10.3	5.3
<i>Boscia angustifolia</i>	4.9 $\pm$ 1.2abcde	6.8 $\pm$ 1.2abc	16.7 $\pm$ 1.2abc	5.4	9.7	6.4
<i>Guiera senegalensis</i>	3.4 $\pm$ 1.2abcdef	8.2 $\pm$ 1.1abc	17.7 $\pm$ 1.2abc	3.8	11.7	6.8
<i>Acacia nilotica</i>	4.1 $\pm$ 1.2abcdef	5.2 $\pm$ 1.1bcd	10.4 $\pm$ 1.1abcde	4.6	7.4	4.0
<i>Acacia sieberiana</i> seed pod	3.5 $\pm$ 1.3abcdef	6.1 $\pm$ 1.2abc	7.8 $\pm$ 1.2abcdef	3.9	8.7	3.0
<i>Cassia siamea</i>	2.5 $\pm$ 1.3bcdefg	2.9 $\pm$ 1.2cde	9.8 $\pm$ 1.2abcdef	2.8	3.1	3.8
Control	0.9 $\pm$ 1.3defg	0.7 $\pm$ 1.3ef	2.6 $\pm$ 1.2fghi	—	—	—

Means in columns followed by the same letter are not significantly different [ $P < 0.05$ ; Ryan–Einot–Gabriel–Welsch Multiple Range Test (SAS Institute, 2003)].

At least some of the *Stomoxys* sp. demonstrated clear preferences to fruit and flowers if given the choice, indicating specific rather than opportunistic sugar-feeding habits. From the selected variety of cultivated and wild fruit that we evaluated, interestingly the seed pods of *P. reticulatum* were the most attractive to all three *Stomoxys* sp. (Table 3). Seed pods of tamarinds, *T. indica*, also attracted significant numbers of *S. niger bilineatus* and *S. sitiens*. The seed pods of both plant species are locally known and used for their high sugar content. Two of the five fruits which attracted significant numbers of *S. sitiens* were the cultivated species of guava, *P. guajava*, and honey melon, *C. melo*. Guava and *F. thonningii* were also the only fruits to significantly attract *S. niger bilineatus*. The fact that the cosmopolitan *S. calcitrans* was attracted significantly to only one local fruit suggests that specificity to local flora is not an indicator of species distribution capability.

The attraction of all three *Stomoxys* species to flowers was more pronounced than their attraction to fruits. This might be a result of the volatiles released by the flowers, the nectar they provide and the potential for increased viability between bloodmeals (Taylor & Berkebile, 2008). In our experiments, the shape and colour of both fruits and flowers were partially masked by the glue traps. Thus in these evaluations, the flies were attracted mainly by the chemical components of these baits. Also, it is not certain if or how much an optical component of attraction has contributed to the results of these experiments.

The fact that the flies were not attracted to all of the flowers could be due in part to a lack of an optical component or flower designs which prevent extraction of nectar by *Stomoxys*. It is worthwhile mentioning that the fruits as well as all of the attractive flowers offer an easy landing platform and the nectar can be easily reached by the flies; however, this is not the case with many of the non-attractive flowers. All of the attractive flowering plants are trees and bushes and most of these bloom continuously, even during the dry season. None of the annuals we evaluated, all of which bloom during the rainy season or shortly thereafter, were attractive to the flies.

Cold anthrone assays of flies caught at both Mopti and the lagoon sites indicate that a greater percentage of sugar-fed flies can be found where suitable nectar sources are readily available. This is supported by the work of Jones *et al.* (1985) who found a higher percentage of sugar-fed *S. calcitrans* at natural beach sites than at dairies in FL, U.S.A. The lagoon site, a natural habitat with numerous flowering and fruited trees, offers many opportunities for sugar. There was nothing comparable between the rice fields at Mopti. Because the availability of blood sources at the two sites was similar, the number of sugar-fed flies caught at the lagoon site indicates that sugar-feeding is a highly important aspect of *Stomoxys* behaviour.

Sugar feeding has been shown to cause decreases in viability and reproduction rates in *S. calcitrans* whereas blood-feeding does much the opposite (Jones *et al.*, 1992). Perhaps the concomitant feeding on sugar and blood acts as a population-regulating mechanism and the effects are determined by the proportions of blood and sugar that are consumed. Hogsette *et al.* (1987) suggested that sugar meals are used to sustain *Stomoxys* populations when bloodmeal availability is low. This probably occurs during certain periods of the year, as the numbers of host animals and nectar sources are subject to seasonal variation. A 12-month study would provide additional knowledge about the relationship of blood and nectar feeding.

When the rhythm of *Stomoxys* feeding activity was evaluated using donkeys and flowers, the fact that sugar feeding is as important in *Stomoxys* ecology as blood-feeding was emphasized once again. All three species were found to have sugar-feeding peaks as high as the morning blood-feeding peaks, but the afternoon blood-feeding period of *S. niger bilineatus* was statistically higher than the sugar-feeding peak. Surprisingly, *S. calcitrans*, which was less responsive in the fruit and flower comparisons, was found to have a numerically higher, but not significantly different, sugar-feeding acrophase than both blood-feeding peaks.

Comparison between the species revealed an interesting temporal separation between *S. calcitrans* and *S. niger bilineatus* for the morning blood-feeding activity, but not during the mid-day sugar-feeding period. *Stomoxys niger bilineatus* was also

shown to have a longer daily activity period, possibly to avoid competition with the other *Stomoxys* species or with predators.

Bimodal blood-feeding activity periods for various *Stomoxys* species can be found in the literature, however, sugar-feeding activity periods have until now been overlooked. Kunz & Monty (1976) and Mihok *et al.* (1996) both reported hourly blood-feeding curves for *S. calcitrans* that are bimodal but of longer duration than we found in Mali. This indicates that these activity periods are dependent upon local conditions.

During the present study, it was observed daily that cattle egrets were picking large numbers of blood-feeding flies during the whole day from cattle. In addition, from the late afternoon until sunset, many thousands of dragon flies (*Brachythemis leucosticta* Burmeister, Libellulidae) were specifically hunting for flies hovering around and feeding on cattle.

The present study provides preliminary data on the attraction of *Stomoxys* species to plant species, demonstrating that they are regular sugar feeders, even when constant blood sources are available. All three *Stomoxys* species were shown to be attracted more to flowers than to fruit. Further studies are needed to better understand the underlying factors at the base of sugar-seeking behaviour. Elucidation of the volatile compounds present in the plants attractive to *Stomoxys* could help to create more efficient traps for both scientific studies and for controlling *Stomoxys* infestations. The fact that three different *Stomoxys* species are attracted to the same plant species, and only to a selected few out of the 54 which were tested, is an encouraging result. Possibly *Stomoxys* control methods might consist of treatment/removal of a few attractive plant species in the areas surrounding rural communities. Fruits could be used to produce bait solutions that could be spiked with oral toxins for application near breeding and resting sites. Similar experiments with several mosquito species and sand flies have been very encouraging.

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