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Field evaluation of BG Sentinel™ traps of four different black-and-white color combinations in Mauritius for enhanced *Ae. albopictus* mosquito collection

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Abstract
The trapping efficacy of four visually modified BG Sentinel™ traps (BGS traps) baited with BG Lure™ were evaluated in three rural (Notre Dame, Panchvati and PDL) and one urban (Curepipe) localities in Mauritius with the aim of determining the most effective trap for *Ae. albopictus* surveillance. *Ae. albopictus* and *Cx. quinquefasciatus* were the dominant mosquito species collected by the BGS traps. The impact of trap color and trap location on *Ae. albopictus* collection differed among localities. In the rural localities, completely black BGS traps collected significantly more *Ae. albopictus* males while in Curepipe, no significant differences were noted among the traps. Moreover, in Notre Dame, completely black BGS traps collected significantly more *Ae. albopictus* females while in the other localities traps' performance did not differ significantly. Hence, field results indicate that completely black BGS traps could be used for an optimized monitoring of *Ae. albopictus* in Mauritius.

Keywords: *Ae. albopictus*, BG Sentinel™ traps, trap color

1. Introduction
As part of an Integrated Vector Management Strategy, the possibility of using the Sterile Insect Technique (SIT) to control *Aedes albopictus* mosquitoes is currently being investigated in two villages in Mauritius (Pointe des Lascars and Panchvati) [1]. The SIT is an environment-friendly species-specific control method which in this case, involves the sustained release of a large number of competitive sterile *Ae. albopictus* males within the target zone which could potentially lead to the biological suppression of its wild population in that area [2].
In an SIT programme, it is very important to establish a robust and sensitive adult surveillance system of the target mosquito for several reasons. Adult incidence data collected during the initial phase of an SIT programme, can be used as a baseline to assess the impact of eventual sterile releases. Moreover, sampling of the adult population can provide important information on the ecology and behavior of the vector mosquito [3] which is essential for planning a sterile release strategy [4]. Finally, to evaluate the effectiveness of a release programme and to make necessary adjustments if required, it is equally important to regularly assess the presence, distribution and proportion of the sterile males released in the field with respect to their wild counterparts by conducting mark-release-recapture experiments [5-7].
Although several traps have been developed to maximize the capture of adult mosquitoes [8], difficulties are often encountered in trapping certain species of *Aedes* mosquitoes, including the *albopictus* species for surveillance purposes [9]. During previous studies carried out mainly in temperate countries [10-13] comparing the effectiveness of commonly-used mosquito traps, the BG Sentinel™ trap (BGS trap, Biogents AG, Regensburg, Germany) was found to be most effective in collecting *Ae. albopictus* mosquitoes which corroborates with results obtained during our preliminary studies in several localities of Mauritius (our unpublished data). However, the standard white BGS trap used in these studies, was not sensitive enough for recapturing released males during mark-release-recapture studies or for detecting the presence of the species during the winter season.
Previously, *Aedes* mosquitoes have been shown to be generally drawn towards highly attractive visual features including alternating black and white stripes, checkerboard patterns, broad black surfaces, and enamel surfaces [14-18]. In the recent study investigating the optical properties of BGS traps in a rural community in Puerto Rico, black BGS traps baited with

BG-Lure captured a significantly greater number of *Ae. aegypti*, *Ae. mediovittatus*, and *Culex quinquefasciatus* mosquitoes than white BGS traps [19]. In addition, the presence of low-reflective (dark) harborage sites in the vicinity of BGS traps have also been shown to negatively affect the recapture rates of *Aedes aegypti* [20]

In this study the standard white BGS trap was visually modified into four BGS traps with different black-and-white color combinations and the trapping efficacy of those traps were evaluated in four localities in Mauritius with the aim of determining the most effective trap for the capture of male *Ae. albopictus* mosquitoes.

2. Materials and Methods

2.1 Experiment Set-up

In March 2015, January 2016, February 2016 and March 2016, a 4 x 4 Latin square experiment were respectively carried out in 4 localities in Mauritius: Notre Dame (S-20^0 8' 22" E-57^0 33' 44"), Curepipe (S-20^0 19' 36" E-57^0 30' 58"), Panchvati (S-20^0 4' 57" E-57^0 41' 31") and Pointe des Lascars (PDL, S-20^0 5' 25" E-57^0 42' 22") to evaluate the attractiveness of BGS traps of four different back-and-white color combinations. Traps 1 and 2 were standard white BGS traps whose white cylindrical body was covered with a shiny black plastic sheet. In Trap 1 (BB, Fig 1a), the white nylon covering was dyed black while in Trap 2 (BW), the white color of the nylon covering was maintained (Fig 1b). Trap 3 (WB, Fig 1c) was a standard BGS trap whose white nylon covering was dyed black and its white cylindrical body maintained. Trap 4 (WW, Fig 1d) served as a control and consisted of an unmodified white BGS trap (i.e. a white cylindrical body and a white nylon covering). All four BGS traps were baited with the BG Sentinel™ lure (Biogents AG, Regensburg, Germany) and a white masking tape (2 cm in width) was wrapped around the outer surface of their intake funnel. Transparent odorless rat glue (Kilzone, Xperia Ltd., Mauritius) was spread onto the masking tape to prevent ants and other crawling insects from accessing the catch bag.

Fig 1: BGS traps with **(a)** a black cylindrical body and black nylon covering; BB, **(b)** a black cylindrical body and white nylon covering; BW **(c)** a white cylindrical body and black nylon covering; WB, and **(d)** a white cylindrical body and white nylon covering; WW.

Four collection sites, located at least 20 m apart, were chosen inside each of the tested locality. In Notre Dame, traps were situated in a forested region while in Panchvati and PDL, traps were placed in yard of inhabitants with some vegetative coverage (which was representative of the region). Traps in Curepipe, were in a relatively more urbanized region (*i.e* in shaded areas, very close to concrete infrastructures). Mean temperature and relative humidity during the trapping period in Panchvati and PDL (1.2 Km from Panchvati), were obtained from a weather station (model Davis Vantage Pro2, Davis Instruments, USA) situated in Panchvati while in Curepipe, data were obtained from a similar weather station within the region. In Notre Dame, climatic data were obtained from a HOBO® (model U14, Onset®, USA) weather data logger placed within the trapping area.

Traps were run on 12 V batteries (31DCXC 12V 130 Ah Deep Cycle Battery, U.S. Battery Manufacturing Company, Corona, CA, USA) and left in operation continuously for four days. Mosquito catch bags were replaced daily at 9 am. and subsequently, the BGS traps were randomly rotated within the different BGS trap stations. The mosquitoes collected were morphological identified under a stereomicroscope. Each experiment was replicated thrice.

2.2 Statistical Analysis

All capture data were transformed with log10 (n + 1) and subsequently tested for normality (Anderson-Darling test) and for homogeneity of variances (Levene's test) prior to statistical analysis. In each locality, the effect of trap color, trap location and the interaction between these two parameters, were evaluated using a 2-way ANOVA for the mean number of (i) *Ae. albopictus* males (ii) *Ae. albopictus* females (iii) *Cx. quinquefasciatus* males and (iv) *Cx. quinquefasciatus* females collected. The *Post hoc* Tukey test was used to test for significant differences between mean numbers of mosquito collected according to the trap color and trap location. Pearson correlation analysis was used to assess the relationship between daily capture rate and the following environmental parameters (daily mean temperature and relative humidity). All statistical analyses were performed using Minitab 16 (Minitab Inc., State College, PA) with alpha level of 0.05. Back-transformed values (mean and 95 % confidence interval) are presented in the text and figures to aid interpretability.

3. Results

In all four localities, *Ae. albopictus* and *Culex quinquefasciatus* were the two major mosquito species collected. Other mosquito species that were collected in low numbers (less than 15 individuals) and therefore were not considered in this study were *Anopheles arabiensis*, *Culex tritaeniorhynchus* and *Aedes fowleri*. In total 1486, 913, 1685 and 501 *Ae. albopictus* mosquitoes were respectively collected in Notre Dame, Curepipe, Panchvati and PDL of which 63.1, 47.0, 35.5 and 53.1% were males. Moreover, 4840, 1187, 1785 and 553 *Cx. quinquefasciatus* mosquitoes were respectively collected in Notre Dame, Curepipe, Panchvati and PDL of which 84.4, 65.5, 40.0 and 50.6 % were males. Mean temperature (±SD) and mean relative humidity (±SD) recorded in Notre Dame, Curepipe, Panchvati and PDL were respectively 26.0 ± 1.0 ^{0}C and 84.4 ± 6.4 %, 25.7 ± 1.0 ^{0}C and 70.1 ± 4.4 %, 27.5 ± 1.1 ^{0}C and 87.8 ± 4.0 % and 27.8 ± 0.6 ^{0}C and 83.9 ± 2.6 %. In all the four localities, there were no significant correlations between the daily capture rate of both mosquito species, mean temperature and mean relative humidity (P> 0.05, Table 1).

Table 2 presents results of ANOVA analyses evaluating the impact of trap color, trap location and the interaction of both parameters on the daily capture of male and female *Ae. albopictus* and *Cx. quinquefasciatus* mosquitoes in the four

study sites. Figures 2, 3, 4 and 5 respectively shows the mean number (95% CI) of *Ae. albopictus* males, *Ae. albopictus* females, *Cx. quinquefasciatus males* and *Cx. quinquefasciatus* females collected daily by the four types of BGS traps in each study site.

Table 1: Pearson correlation coefficient (r^2) between meteorological data (daily mean of temperature and relative humidity) and daily capture rate of *Ae. albopictus and Cx. quinquefasciatus* mosquitoes from BGS traps in four localities in Mauritius (Notre Dame, Curepipe, Panchvati and PDL).

Localities	Meteorological variables	Daily capture rate			
		Ae. albopictus		*Cx. quinquefasciatus*	
		r^2	P	r^2	P
Notre Dame	Average temperature	0.3	0.348	0.24	0.458
	Relative Humidity	-0.37	0.236	-0.36	0.25
Curepipe	Average temperature	0.05	0.877	-0.12	0.716
	Relative Humidity	0.07	0.837	0.36	0.251
Panchvati	Average temperature	-0.02	0.94	0.01	0.986
	Relative Humidity	0.06	0.865	0.29	0.368
PDL	Average temperature	0.21	0.507	0.19	0.554
	Relative Humidity	0.37	0.231	0.157	0.626

Table 2: Statistical parameters (*F*, df and *P* values) of 2 way-ANOVA analyses carried out to evaluate the effect of trap color, trap location and the interaction between trap color and trap location on the mean number of *Ae. albopictus* and *Cx. quinquefasciatus* mosquitoes collected daily by BGS traps of four different colors in four localities in Mauritius (Notre Dame, Curepipe, Panchvati and PDL).

Locality	Mosquito collected		Trap color	Trap location	Trap color and trap location
	Species	Sex	F, P (df = 3,32)	F, P (df = 3,32)	F, P (df = 9,32)
Notre Dame	*Ae. albopictus*	M	65.02, < 0.001	4.31, 0.012	6.12, <0.001
	Ae. albopictus	F	58.73, <0.001	3.16, 0.038	6.84, <0.001
	Cx. quinquefasciatus	M	237.22, <0.001	50.18, <0.001	4.68, 0.001
	Cx. quinquefasciatus	F	18.12, <0.001	11.99, <0.001	4.06, 0.002
Curepipe	*Ae. albopictus*	M	2.13, 0.116	0.12, 0.948	0.43, 0.911
	Ae. albopictus	F	0.55, 0.653	1.42, 0.256	0.37, 0.939
	Cx. quinquefasciatus	M	35.07, <0.001	0.10, 0.957	1.55, 0.174
	Cx. quinquefasciatus	F	14.30, <0.001	0.94, 0.431	0.54, 0.837
Panchvati	*Ae. albopictus*	M	18.07, <0.001	37.04, <0.001	2.46, 0.029
	Ae. albopictus	F	2.03, 0.129	10.3, <0.001	1.71, 0.128
	Cx. quinquefasciatus	M	1.40, 0.262	1.09, 0.368	0.96, 0.491
	Cx. quinquefasciatus	F	1.59, 0.212	10.65, <0.001	2.20, 0.049
PDL	*Ae. albopictus*	M	53.32, <0.001	26.01, <0.001	2.09, 0.061
	Ae. albopictus	F	0.41, 0.747	2.15, 0.114	1.14, 0.366
	Cx. quinquefasciatus	M	0.83, 0.487	0.47, 0.708	1.30, 0.277
	Cx. quinquefasciatus	F	1.95, 0.142	46.82, <0.001	14.57, <0.001

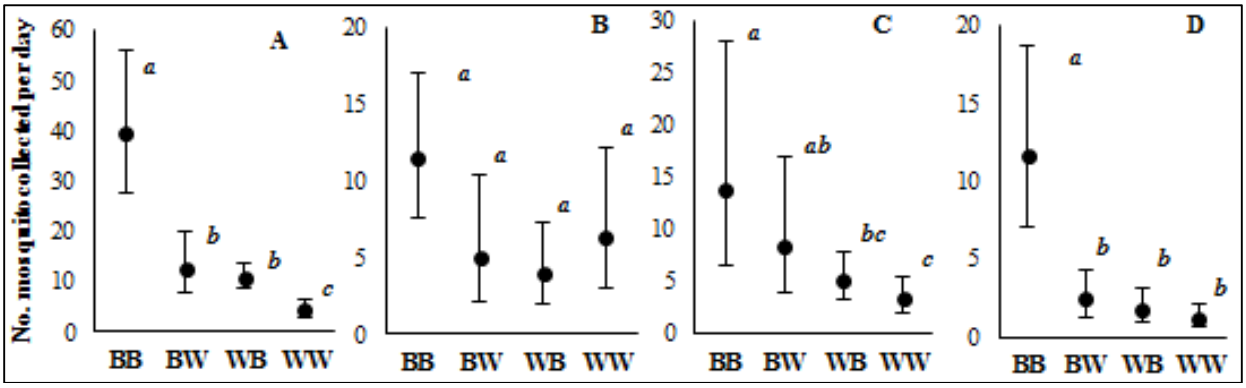

Fig 2: Daily capture rates of *Ae. albopictus* males by BGS traps of four different black-and-white color combinations (BB, BW, WB and WW) in (A) Notre Dame, (B) Curepipe, (C) Panchvati and (D) PDL. All traps were baited with BG Lure. Mean value and 95% confidence limits of back-transformed data are reported. Different letters represent statistical differences between treatments ($P < 0.05$).

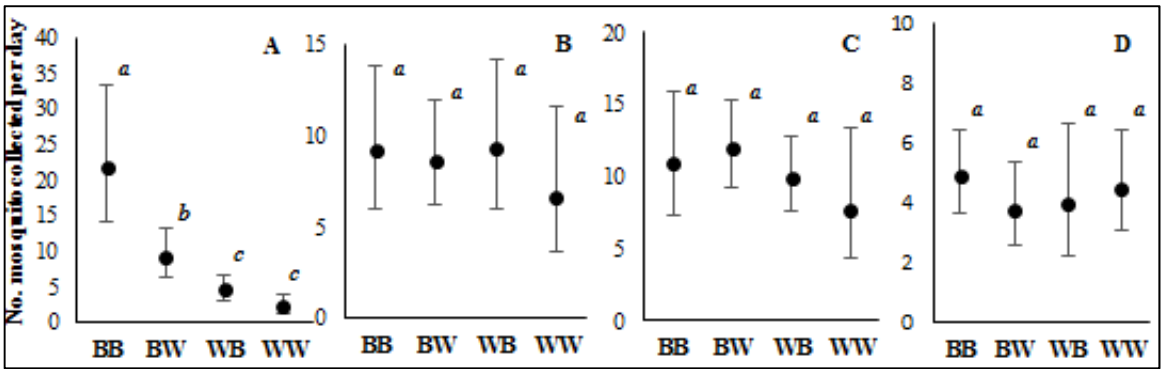

Fig 3: Daily capture rates of *Ae. albopictus* females by BGS traps of four different black-and-white color combinations (BB, BW, WB and WW) in (A) Notre Dame, (B) Curepipe, (C) Panchvati and (D) PDL. All traps were baited with BG Lure. Mean value and 95% confidence limits of back-transformed data are reported. Different letters represent statistical differences between treatments ($P < 0.05$).

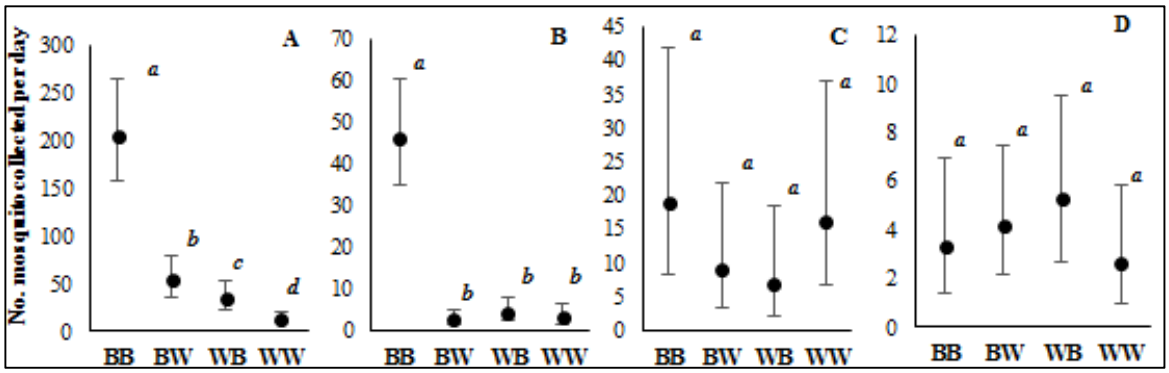

Fig 4: Daily capture rates of *Cx. quinquefasciatus* males by BGS traps of four different black-and-white color combinations (BB, BW, WB and WW) in (A) Notre Dame, (B) Curepipe, (C) Panchvati and (D) PDL. All traps were baited with BG lure. Mean value and 95% confidence limits of back-transformed data are reported. Different letters represent statistical differences between treatments ($P < 0.05$)

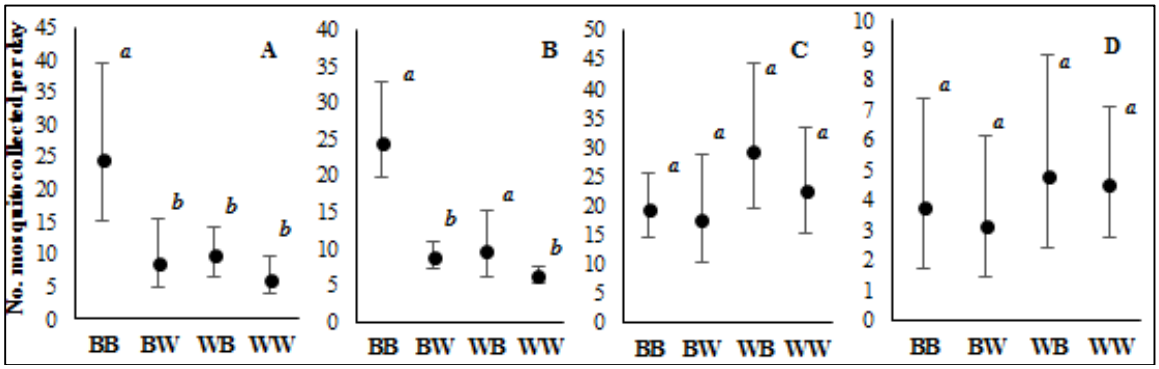

Fig 5: Daily capture rates of *Cx. quinquefasciatus* females by BGS traps of four different black-and-white color combinations (BB, BW, WB and WW) in (A) Notre Dame, (B) Curepipe, (C) Panchvati and (D) Pointe des Lascars. All traps were baited with BG lure. Mean value and 95% confidence limits of back-transformed data are reported. Different letters represent statistical differences between treatments ($P < 0.05$).

3.1 Notre Dame

In Notre Dame, trap color and trap location significantly affected the capture rate of male and female *Ae. albopictus* and *Cx. quinquefasciatus* mosquitoes. Completely black BGS traps (BB) collected significantly more mosquitoes of both species than the other traps. A significant interaction between trap color and trap location on mosquito capture was also noted in Notre Dame.

3.2 Curepipe

In Curepipe, trap color and trap location had no effect on the capture rates of male and female *Ae. albopictus*. Moreover, while trap color had no significant effect on the capture rates of male and female *Cx. quinquefasciatus*, their collection was significantly influenced by trap location.

3.3 Panchvati

In Panchvati, trap color and trap location significantly affected the number of *Ae. albopictus* males collected and there was a significant interaction between both parameters. More *Ae. albopictus* males were collected from traps having a black cylindrical body (BB and BW traps). Moreover,

significantly more number of *Ae. albopictus* males were collected in a location heavily planted with mango trees.
Unlike trap color, trap location significantly affected the number of *Ae. albopictus* females collected where more females were collected in two locations which were in very close proximity to cattle and goat enclosures.
Trap color did not significantly influence the collection of male and female *Cx. quinquefasciatus.* However, trap location had a significant effect on the capture of *Cx. quinquefasciatus* females.

3.4 PDL
In PDL, trap color and trap location significantly affected the number of *Ae. albopictus* males collected and a significant interaction between both parameters was noted. Completely black BGS traps (BB) collected significantly more males than the other traps. The capture rates of *Ae. albopictus* females and *Cx. quinquefasciatus* males were not significantly affected by trap color and trap location. Moreover, trap location affected the capture of *Cx. quinquefasciatus* females but not trap color. Furthermore, the interaction between trap color and trap location significantly impacted female collection.

4. Discussion
In this study, *Ae. albopictus* and *Cx. quinquefasciatus* were the dominant mosquito species collected by the BGS traps. The impact of trap color and trap location and whether or not there was a significant interaction between both parameters on the capture rate of the two species, varied greatly according to the study sites. The geographical features and micro-climatic environment of the BGS trap location, are two factors that could probably have elicited this differential response to trap stimulus. Accordingly, while the presence and color of objects near white BGS traps have been found to significantly influence the capture of female *Ae. aegypti* [20], it was also observed in another study that light intensity, temperature and relative humidity within the immediate vicinity of BGS traps could significantly affect the collection of *Ae. albopictus* mosquitoes [21].
In Notre Dame, Panchvati and PDL where traps were usually found in close proximity to vegetation, completely black BGS traps (BB traps) attracted significantly more *Ae. albopicus* males than the other traps and a significant interaction between trap color and trap location on male capture was also observed. The propensity of black BGS trap to attract more mosquitoes, was also demonstrated during a study in Puerto Rico where black BGS traps captured significantly more *Ae. mediovittatus*, *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes than white BGS traps [19]. Moreover, in line with our observations, location of BGS trap was found to significantly affect the collection of *Ae. albopictus* mosquitoes during a study investigating the micro-environment of BGS traps in New Jersey [21] and in another study evaluating BGS trap as a management tool for *Ae. albopictus* in Italy [22].
Although a thorough evaluation of trap location was not the focus of this study, it is worth noting that in Panchvati and PDL, more *Ae. albopictus* males were collected from traps situated very close to mango and letchi trees. Considering the fact that small swarms of *Ae. albopictus* mosquitoes were often observed near the trunk of those two fruit trees during trap collections and routine entomological surveys conducted in those villages (our unpublished data) and that past studies have demonstrated the differential preference of numerous mosquito species for certain host plants [23-28]; in-depth studies could be conducted to investigate the behavioral responses of *Ae. albopictus* males to scent compounds isolated from those two plants with the aim of developing an artificial bait that could be supplemented to mosquito traps to enhance their male trapping efficacy.
Unlike in males, trap color did not elicit a significant response from *Ae. albopictus* females in Curepipe, Panchvati and PDL, which concords with observations made by Ball and Ritchie [20] who noted a more pronounced effect in *Ae. aegypti* males' response to dark harborage sites near BGS traps than in females. In Notre Dame however, completely black BGS traps collected significantly more *Ae. albopictus* females than the other traps. The experiment being conducted in a forested area in Notre Dame, it is believed that females from all physiological stages were in close proximity to the traps and hence differed in their responses to the latter. In contrast, traps in the other localities were very close to inhabitations and could therefore have collected mostly host-seeking females which were primarily attracted to the traps by the artificial human skin odors emitted by the BG Lure [3, 29, 30]. BG-Lure has in fact been found to effectively attract female *Ae. aegypti* [3, 31] and *Ae. albopictus* [12] while during a study in Brazil [32], BGS traps baited with BG Lure captured significantly more *Ae. aegypti* host-seeking females than females in other behavioral and physiological stages. Further studies therefore need to be carried out to investigate the correlation between capture rates of females with different physiological status in locations with distinct ecological characteristics in Mauritius.
Although *Cx. quinquefasciatus* is usually a nocturnal biter [33, 34], it was noted that in Notre Dame and Curepipe, significantly more males and females of the species were attracted to completely black BGS traps. A similar observation was made by Barrera *et al.* [19] in Puerto Rico where black BGS traps attracted significantly more *Cx. quinquefasciatus* mosquitoes than white ones. Moreover, in a study by Wilton and Kloter [35] black cylinder traps were found to be particularly attractive to *Cx. quinquefasciatus* mosquitoes. Although *Cx. quinquefasciatus* mosquitoes was not the main species under investigation in this study, the high number of the mosquito collected from BGS traps in all the four localities, indicates the possibility of using the trap as a surveillance tool for the mosquito species which is a potential vector of filariasis in Mauritius. Furthermore, the high incidence of *Cx. quinquefasciatus* observed in Panchvati and PDL (pilot sites for an SIT programme against *Ae. albopictus*), calls for a proper sensitization of the residents in the two villages to the probability that they might still experience biting nuisances from *Cx. quinquefasciatus* despite an eventual suppression of *Ae. albopictus* mosquitoes during releases of sterile males in the area.

5. Conclusion
In this study during which four visually modified BGS traps baited with BG Lure were tested, the trapping efficacy of completely black BGS traps was significantly higher than the other traps for male *Ae. albopictus* mosquitoes in Notre Dame, Panchvati and PDL, for female *Ae. albopictus* mosquitoes in Notre Dame and for male and female *Cx.*

quinquefasciatus mosquitoes in Notre Dame and Curepipe while it did not significantly differ from the other traps in all the other tested conditions. Completely black BGS traps could hence be used as a monitoring tool for an optimized surveillance of *Ae. albopictus* and *Cx. quinquefasciatus* mosquitoes in Mauritius. Moreover, in some study sites, trap location significantly affected the capture of *Ae. albopictus* and *Cx. quinquefasciatus* mosquitoes, thereby highlighting the importance of suitable site selection specific to the study area, for the placement of BGS traps to ensure an accurate monitoring of the species' incidence in the tested locality.

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