Effect of zebra skin-derived compounds on field catches of the human African trypanosomosis vector *Glossina fuscipes fuscipes*

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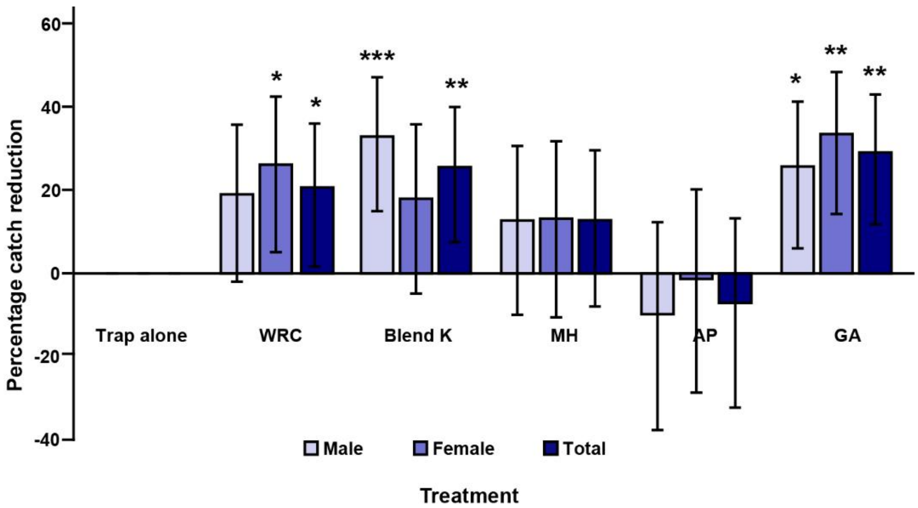
Highlights

- Zebra skin derived blend K repelled riverine tsetse flies *Glossina fuscipes fuscipes*.
- Sex-specific variations occur in the repellent effect of blend K and WRC.
- Geranyl acetone accounts for the repellent effect of blend K on *G. f. fuscipes.*
- Olfactory cues are important in the bioecology of riverine tsetse flies.

Graphical abstract

Zebra skin-derived blend K and its component, geranyl acetone, repel *Glossina fuscipes fuscipes* showing their potential for use in the control of these medically-important tsetse flies.



Abstract

The riverine tsetse fly *Glossina fuscipes fuscipes* is a major vector of trypanosome pathogens causing African trypanosomiasis. This fly species uses a combination of olfactory and visual cues to locate its hosts. Previously, traps and targets baited with visual cues have been used in vector control, but the development of olfactory-based tools has been challenging. Recently, repellents have shown promise as olfactory-based tools in tsetse vector control. Here, we evaluated a three-component blend comprising 6-methyl-5-hepten-2-one, acetophenone and geranyl acetone (blend K), previously identified as a repellent for savannah tsetse flies in zebra skin odor, on *G. f. fuscipes* populations. Using a series of 6 × 6 randomized Latin square designed experiments, *G. f. fuscipes* catches in biconical traps were monitored on four islands of Lake Victoria in western Kenya between July and September 2019, after the long rainy season. Traps were baited with blend K and individual components of this blend. The known tsetse repellent blend WRC (waterbuck repellent compounds) and trap alone were included as controls. Daily catch data in thirty-six replicate trials were analyzed using generalized linear model with negative binomial error structure using the package "MASS" in R. Treatment, day and site were set as predictor variables. Our results showed that, blend K significantly reduced *G. f. fuscipes* catches by 25.6% ($P < 0.01$) compared to the control trap alone but was not significantly different from WRC which reduced catches by 20.7% ($P < 0.05$). Of the individual compounds, geranyl acetone solely significantly reduced catches by 29.1% ($P < 0.01$) which did not differ from blend K or WRC. We conclude that geranyl acetone accounts for the repellent effect of blend K on the riverine tsetse fly, *G. f. fuscipes*, demonstrating the ecological importance of animal skin odors in the host-seeking behavior of medically important tsetse fly vectors.

Keywords: Riverine tsetse fly; sleeping sickness; repellent blend; zebra skin odor; Lake Victoria

1. Introduction

Tsetse flies (*Glossina* species), nicknamed “The Poverty Fly” transmit Human African trypanosomiasis (HAT), a deadly neglected tropical disease commonly called sleeping sickness (Alsan, 2015; Holmes, 2014; Sutherland and Tediosi, 2019). Despite recent successes in the disease control and elimination as a public health problem (Akazue et al., 2019), unreported or undiagnosed cases, aparasitaemic and asymptomatic human reservoirs, and possible unknown animal reservoirs (Büscher et al., 2018; Capewell et al., 2019; Simo and Rayaisse, 2015; Sudarshi et al., 2014) threaten the sustainability of these achievements. An integrated approach, combining medical interventions and vector control, is therefore required for a cost effective, and sustained elimination of HAT (Courtin et al., 2015; Mahamat et al., 2017; Rock et al., 2017; Sutherland et al., 2017).

Tsetse flies are found only in Africa and comprise 31 extant species and subspecies placed in three groups: the *Morsitans* or savannah, the *Fusca* or forest and the *Palpalis* or riverine groups (Akazue et al., 2019; Bouyer et al., 2019). The riverine tsetse flies are the key drivers of the transmission of the chronic, endemic Gambian HAT (implicated in more than 97% of overall reported cases of sleeping sickness) (Simarro et al., 2008; Tirados et al., 2015; WHO, 2015). *Glossina fuscipes* subspecies are responsible for the highest number of HAT cases (about 90%), and *G. f. fuscipes* is the most important in terms of the disease transmission and distribution (Gloria-Soria et al., 2018; Omolo et al., 2009; Simarro et al., 2008; Tirados et al., 2015). Where they are present, particularly in West Africa and Uganda, *G. f. fuscipes* is also an important vector of trypanosome pathogens causing African trypanosomiasis in livestock (also called nagana in cattle) (Beadell et al., 2010; FAO 1992a; Opiro et al., 2017). Compared to the Morsitans (savannah) group, tsetse flies in the Palpalis (riverine) group are less responsive to host odors (Oloo et al., 2014; Torr and Vale, 2015; Vale et al., 2014). As such, trapping has mostly exploited visual cues. Visual attractive devices called tiny targets (0.5 m × 0.25 m dimension insecticide impregnated blue fabric and black netting material) have been shown to reduce riverine tsetse fly densities by over 90 % (Lindh et al., 2009; Tirados et al., 2015; Rayaisse et al., 2011; Rock et al., 2017). Despite its cost effectiveness, a major limitation of this vector control method is that the tiny targets are not maintained post-interventions (Bouyer et al., 2009). Yet tsetse populations could recover even after more than 90% reduction (Mbewe et al., 2018), showcasing the need to complement the use of tiny targets with new vector control methods.

A recent innovative vector control strategy is the tsetse repellent technology (Bett et al., 2015; Saini et al., 2017). This technology, originally developed for the control of savannah tsetse flies, employs potent synthetic or natural repellents (identified from non-preferred vertebrate hosts such as waterbuck and zebra) to disrupt the vector-host contact as well as the disease transmission cycle. A four-component repellent blend identified from waterbuck, *Kobus ellipsiprymnus defassa* (waterbuck repellent compounds, WRC), comprising geranyl acetone, guaiacol, pentanoic acid and δ-octalactone embedded in repellent collars is currently being used to protect cattle against savannah tsetse flies in some parts of Kenya (Saini et al., 2017). Further field evaluation of the WRC on the riverine tsetse flies *G. f. fuscipes*, using traps and targets, has shown some promising results for the control of the vectors of the human-infective trypanosomes (Mbewe et al., 2019).

A three-component repellent was recently identified for the savannah tsetse flies, *Glossina pallidipes*, in zebra skin odor (Olaide et al., 2019). This three-component repellent blend comprising the ketones 6-methyl-5-hepten-2-one, acetophenone and geranyl acetone, mixed in their natural ratio of occurrence in zebra skin, represents an alternative repellent blend for savannah tsetse flies (Olaide et al., 2019). However, its effects on riverine tsetse flies particularly *G. f. fuscipes*, which are key vectors of HAT, and potential for use in disease control are still unknown. We hypothesized that the three-component blend (referred to as blend K) has a repellent effect on *G. f. fuscipes*. We tested this by comparing the field performance (trap catches of *G. f. fuscipes*) of blend K and its individual components with those of WRC.

2. Methods

2.1. Study area

This study was carried out from July to September 2019 on four islands of Lake Victoria Homabay County, western Kenya (Fig. 1). The islands included small Chamaunga (0.2 km^2; 1 km circumference), big Chamaunga (0.2 km^2; 1.5 km circumference), Rusinga (43km^2) and Manga (1 km^2) islands (Esterhuizen et al., 2011; Tirados et al., 2015). Unlike Rusinga (connected to the mainland by a 100 m causeway) and Manga islands, the Chamaunga islands are not inhabited by humans (Omolo et al., 2009). Rainfall patterns in the study area are bimodal, long rains occur from

March to May and short rains from October to December (Manangwa et al., 2017). The experiments were carried out after the long rainy season. The vegetation cover on the islands is predominantly *Aeschynomene eraphyroxylon* (freshwater mangroves), *Lantana camara* (tickberry), and *Dombeya spp*. (tropical hydrangea) (Mbewe et al., 2018; Tirados et al., 2015). *Glossina f. fuscipes* is the only tsetse fly species that occupies the islands and it mainly feeds on *Varanus niloticus* (monitor lizards) but also on *Hippopotamus amphibious* (hippopotamus), livestock and humans (Tirados et al., 2015; Wamwiri et al., 2007).

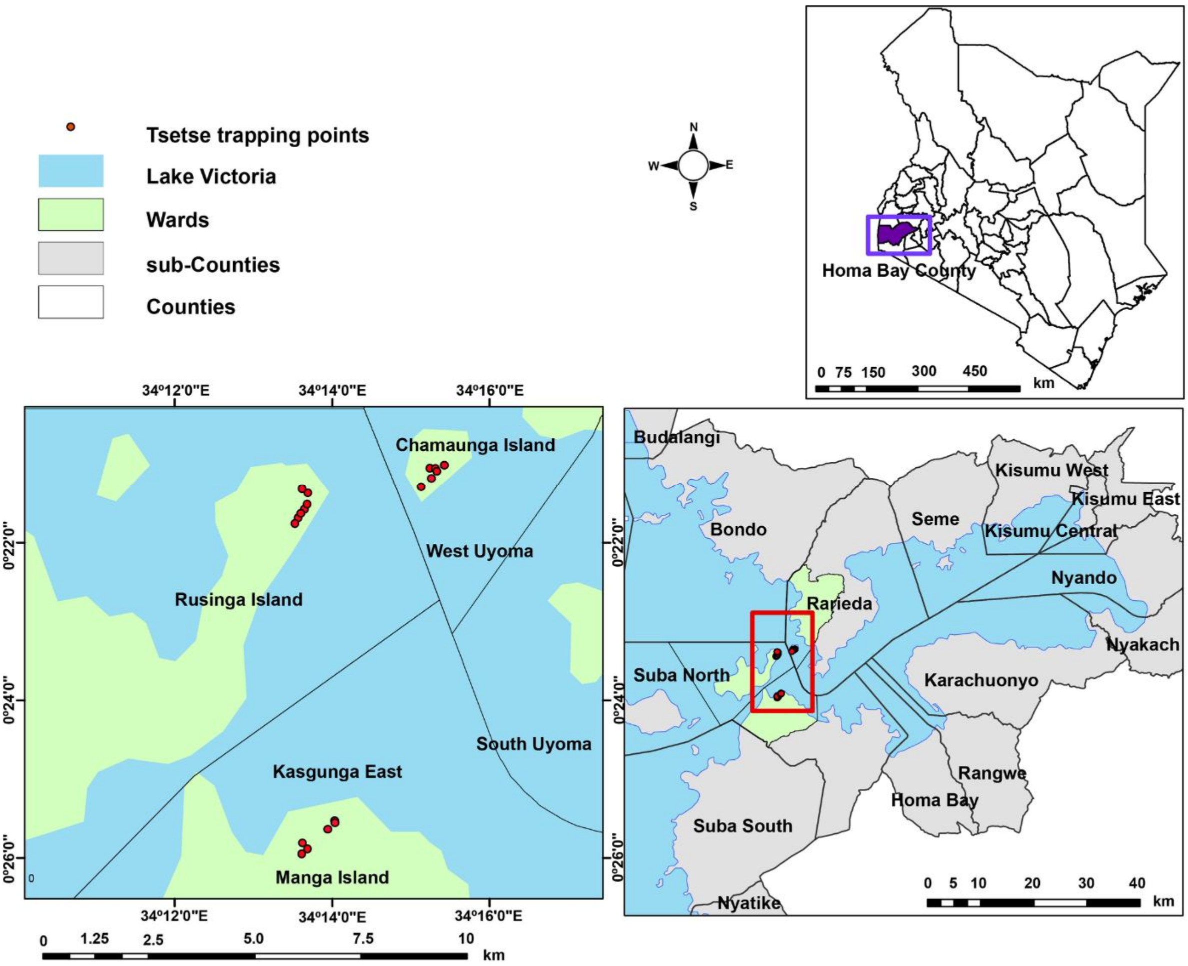


Fig. 1. Map showing the tsetse trapping points on islands of Lake Victoria Homa Bay County Kenya

2.2. Traps

Eighteen biconical traps (100% polyester, Vestergaard Frandsen, Switzerland) described by Challier and Laveissiere (1973) were used throughout this study. The traps were placed at open sites along the forest gallery on the shores of each of the islands. *Glossina. fuscipes fuscipes* is known to be abundant at these sites (Mbewe et al., 2018) for field evaluation of semiochemicals. Inter-trap distance on each island was about 150 m. Treatment chemicals were placed 15-20 cm downwind of the traps.

2.3. Test compounds and dispensers

The chemicals used, included: 6-methyl-5-hepten-2-one (99%, M48805, Sigma-Aldrich), acetophenone (≥ 99%, 00790, Sigma-Aldrich), geranyl acetone (65% geranyl acetone and 35% neryl acetone, 270716, Sigma-Aldrich). The zebra skin-derived ketone blend (blend K) of 6-methyl-5-hepten-2-one, acetophenone and geranyl acetone was constituted in the ratio 2:4:3 to simulate their natural proportions in zebra skin (Olaide et al., 2019). Like the known tsetse repellent blend, WRC (waterbuck repellent compounds), blend K and individual compounds were dispensed from sealed polyethylene sachets (0.125 mm thickness, Audion Elektro, Derby, UK) folded into a tetrahedral shape (Mbewe et al., 2019; Olaide et al., 2019). Each sachet contained 4.5 ml of the respective blend or individual chemicals, and two sachets were used per trap. Two sachets of each treatment previously shown as optimum repellent doses were used for the respective traps (Mbewe et al., 2019; Olaide et al. 2019). Freshly prepared sachets of the individual compounds and blends were used for each replicate of the 6 × 6 Latin square-designed experiment. The same sachets were maintained for the duration of each replicate of the experiment.

2.4. Experimental design

The experiment followed a 6×6 Latin square design (FAO 1992b; Olaide et al., 2019). The six treatments tested included: biconical trap alone (control), biconical trap with WRC, biconical trap with blend K (three-component ketone blend mimicking natural ratios in zebra skin odor), biconical trap with 6-methyl-5-hepten-2-one, biconical trap with acetophenone, and biconical trap with geranyl acetone. The 6 × 6 Latin square was randomized by row, followed by column, according to standard procedures (FAO 1992b), to give the final randomized Latin square used in the experiment (Table S1). Treatments were moved to new positions daily according to the randomized Latin square. Three 6 × 6 Latin square experiments ran concurrently, one on each island. In total, there were six replicates of the 6 × 6 Latin square-designed experiment and thirty-six data points for each treatment. Trap catches were harvested daily between 06:30 and 08:30 hrs. Daily trap catches were sorted based on sex, counted and recorded.

2.5. Release rates from sachets

The average release rates of each treatment (blend and individual compounds) from the sealed polyethylene sachets were measured by weighing the sachets before and after use in experiments to reflect the actual scenario in a field setting. From the difference in weights, the release rates, in mg/hr, were calculated from nine individual sachets per treatment.

2.6. Data analyses

All data analyses were carried out in R software v 3.5.2, using the graphic user interface R studio v 1.2.5019. To test the effect of each treatment on catches of *G. f. fuscipes*, the daily catch data (response variable) were subjected to a generalized linear model with negative binomial error structure; day, site (island) and treatment were set as factors (predictor variables) executed using the package 'MASS' (Venables and Ripley, 2002). To visualize the variations in catches for each treatment actual traps catches were used to generate box plots. In order to see the detailed distributions of the catch data, dot plots were superimposed on the box plots. Significance level for all tests were set at $\alpha < 0.05$.

2.7. Ethics statement

The International Centre of Insect Physiology and Ecology (*icipe*) and owners of the islands on which we carried out our studies, gave consent to our activities.

3. Results

3.1. Effects of blend K and individual ketones on trap catches

Overall, 3,646 *G. f. fuscipes* (2,005 males and 1,641 females) were caught in traps during our experiments. Blend K, identified in zebra skin, significantly reduced field catches of *G. f. fuscipes* in biconical traps (25.6% mean catch reduction, 95% CI: 7.5 - 40.1%) compared to the trap alone (control) (Fig. 2, Table 1). The known repellent blend WRC caused a significant reduction in catches compared to the control (20.7% mean catch reduction, 95% CI: 1.7 – 36.1%). Among the individual compounds evaluated, only geranyl acetone significantly reduced trap catches of *G. f. fuscipes* (29.1% mean catch reduction, 95% CI: 11.8 – 43.1%) (Table 1, Fig. 3). This repellent effect on *G. f. fuscipes* was not significantly different from blend K and WRC. While the ketone 6-methyl-5-hepten-2-one reduced trap catches by only 13.6% (CI: -8.0 – 29.6%), acetophenone increased catches by 1.81% (CI: -32.4 – 13.2%). Catches from both compounds were not significantly different from those in the biconical trap alone (Table 1, Fig. 3).

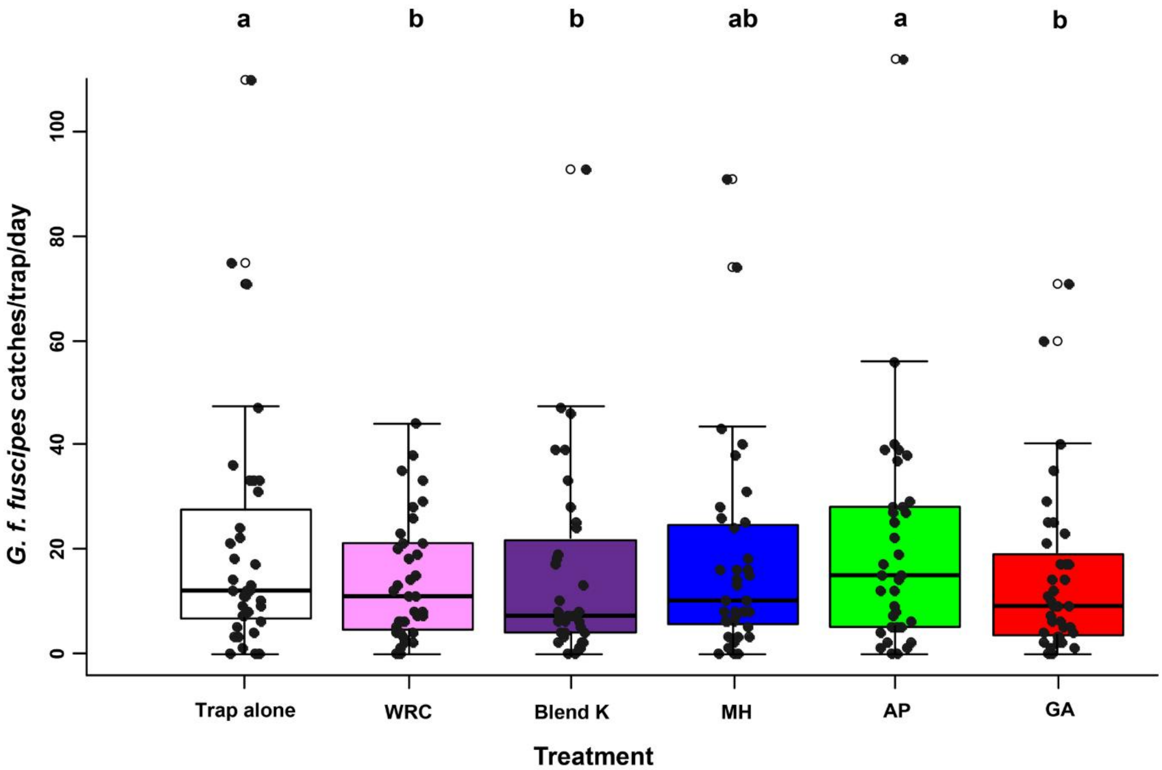


Fig. 2. Daily catches of *G. f. fuscipes* in biconical traps combined with different treatments. Boxplot shows the actual daily count data of pooled male and female catches per trap for each treatment. The top and bottom lines of each box represent the third and first quartiles respectively, and the bold black line across the middle of the box is the median catch for each treatment. The boundaries of the box plot whiskers are the minimum and maximum catches while values outside these boundaries are outliers. Unshaded dots outside the boundaries represent overlapping outliers. Experiment followed a 6 × 6 Latin square design in 36 replicates for each treatment. Trap alone, control; MH, 6-methyl-5-hepten-2-one; AP, acetophenone; GA, geranyl acetone; Blend K, three-component blend of MH, AP and GA formulated in their natural ratio of occurrence in zebra skin. Boxes with the same letters are not significantly different from each other (GLM, $P < 0.05$).

Table 1

Mean catches (± SEM) of *G. f. fuscipes* in biconical traps combined with different treatments

Treatment	Male			Female			Total		
	Mean (± SEM)	**Catch index (95% CI)**	***P*-value**	**Mean (± SEM)**	**Catch index (95% CI)**	***P*-value**	**Mean (± SEM)**	**Catch index (95% CI)**	***P*-value**
Trap alone	11.19 ± 2.16			8.81 ± 1.70			20 ± 3.79		
WRC	8.28 ± 1.26	0.81 (0.64-1.02)	0.073	5.72 ± 0.91	0.74 (0.57-0.95)	0.017	14 ± 1.96	0.79 (0.64-0.98)	0.033
Blend K	7.47 ± 1.43	0.67 (0.53-0.85)	< 0.001	7.75 ± 2.07	0.82 (0.64-1.05)	0.113	15.22 ± 3.16	0.74 (0.60-0.92)	0.007
MH	9.03 ± 1.49	0.87 (0.69-1.10)	0.244	8.25 ± 2.00	0.87 (0.68-1.11)	0.252	17.28 ± 3.32	0.87 (0.70-1.08)	0.204
AP	11.14 ± 1.68	1.10 (0.88-1.38)	0.408	9.22 ± 2.06	1.01 (0.80-1.29)	0.913	20.36 ± 3.58	1.07 (0.87-1.32)	0.516
GA	8.58 ± 1.65	0.74 (0.59-0.94)	0.013	5.83 ± 1.09	0.66 (0.51-0.86)	0.002	14.42 ± 2.69	0.71 (0.57-0.88)	0.002

Experiments were carried out in a 6 × 6 Latin square design with 36 replicates for each treatment. CI, confidence interval; MH, 6-methyl-5-hepten-2-one; AP, acetophenone; GA, geranyl acetone; Blend K, three-component blend of MH, AP and GA formulated in their natural ratio of occurrence in zebra skin. *P*-values < 0.05 are statistically significant.

Trap catches of males (59.5 %; 95% CI: 50.9-62.6%) were significantly higher than females (40.5%; 95% CI: 39.2-48.5%). Sex-specific reduction in catches was observed for blend K and WRC relative to the control. Blend K significantly reduced male catches (percentage catch reduction 33.0%, 95% CI: 15.0 – 47.2%), with a similar effect observed for females only for WRC (percentage catch reduction 26.2%, 95% CI: 5.1 - 42.6%) (Table 1, Fig. 3). Geranyl acetone significantly reduced catches of both sexes (male: percentage catch reduction 25.8%, 95% CI: 6.0 – 41.4%; female: percentage catch reduction 33.5%, 95% CI: 14.3 – 48.5%) catches. Neither 6-methyl-5-hepten-2-one nor acetophenone had any effect on male and females catches compared to the control (Table 1, Fig. 3).

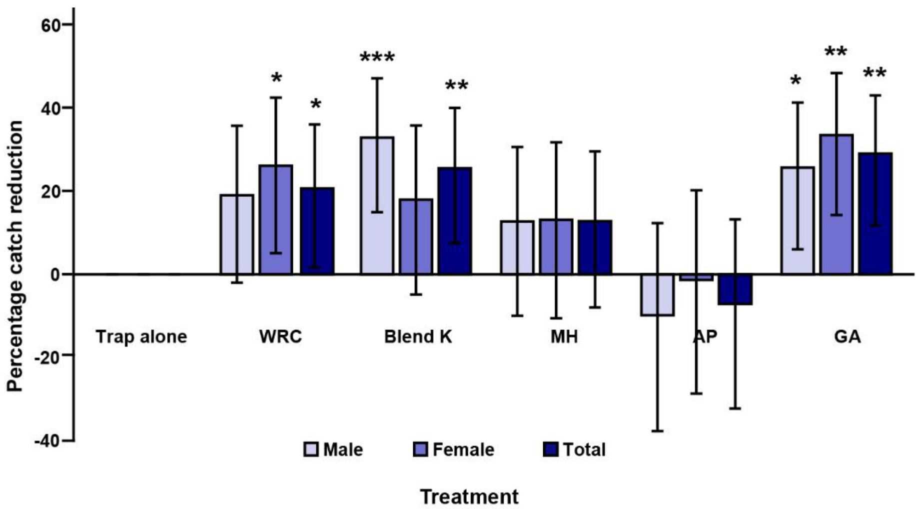


Fig. 3. Percentage catch reduction of *G. f. fuscipes* in biconical traps combined with different treatments. Trap alone, control; MH, 6-methyl-5-hepten-2-one; AP, acetophenone; GA, geranyl acetone; Blend K, three-component blend of MH, AP and GA formulated in their natural ratio of occurrence in zebra skin Values on the upper part of the graph correspond to reduction in catches for each treatment compared to the control, while those on the lower part indicate an increase in catch compared to the control. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$. Error bars show 95% confidence interval of the percentage catch reduction. Results were obtained from 36 replicates per treatment.

3.2. Release rates from sachets

The release rate of 6-methyl-5-hepten-2-one was the highest (4.04 mg/hr), while geranyl acetone was the lowest (0.73 mg/hr) (Table 2). The release rate of blend K (3.08 mg/hr) did not differ from that of acetophenone (3.33 mg/hr), although significantly higher in comparison to that of WRC (1.03 mg/hr) and geranyl acetone (0.73 mg/hr). The release rates of blend K and acetophenone were three-fold higher compared to that of either WRC or geranyl acetone (Table 2).

Table 2
Mean release rates of individual compounds and blends from each sachet and *G. f. fuscipes* catch (± SEM)

Compound/Blend	Mean release rates (mg/hr)*	Mean catch*
WRC	1.03 ± 0.02^{a}	14 ± 1.96^{a}
Blend K	3.08 ± 0.08^{b}	15.22 ± 3.16^{a}
6-methyl-5-hepten-2-one	4.04 ± 0.18^{c}	17.28 ± 3.32^{ab}
Acetophenone	3.33 ± 0.06^{b}	20.36 ± 3.58^{b}
Geranyl acetone	0.76 ± 0.05^{a}	14.42 ± 2.69^{a}

Mean release rates were obtained from 9 replicates of each compound or blend. Mean *G. f. fuscipes* catch denotes means of pooled male and female catches. Means with the same letter in superscript in the same column are not significantly different (ANOVA followed by SNK post hoc test; $P < 0.05$). Blend K, three-component blend of 6-methyl-5-hepten-2-one, acetophenone and geranyl acetone formulated in their natural ratio of occurrence in zebra (*Equus quagga*) skin.

4. Discussion

Our findings showed that the zebra skin-derived blend K, comprising 6-methyl-5-hepten-2-one, acetophenone and geranyl acetone repelled the riverine tsetse fly *G. f. fuscipes*. Notably, of the individual components of blend K, geranyl acetone duplicated the repellent effect of blend K. Mbewe et al. (2019) recently demonstrated a similar repellent effect of geranyl acetone and WRC on this tsetse fly species. Additionally, geranyl acetone has been found to contribute to the antifeedant effect of WRC on *G. f. fuscipes* (Diallo et al., 2020), confirming its parsimonious role in the bioecology of tsetse flies. Thus, our findings support the crucial role of olfaction in the bioecology of this riverine tsetse fly species more than previously known (Oloo et al., 2014; Torr and Vale, 2015). The repellents identified in this study could potentially be used in integrated tsetse control in the form of a ‘push (e.g. repellent) – pull’ (e.g, insecticide-treated targets) strategy as part of elimination efforts of sleeping sickness.

The responsiveness of *G. f. fuscipes* to these repellent compounds is intriguing and suggests a common perception pathway in both fly groups. Olfactory receptors (ORs) are involved in the reception of odorants and they mediate decoding of specific behavioral responses in hematophagous insects such as tsetse flies (e.g. host seeking and avoidance) (Chada et al., 2019; Ghaninia et al., 2007; Macharia et al., 2016; Vieira et al., 2007). The putative ORs in *G. f. fuscipes* responsible for coding the components of the known repellent WRC have recently been predicted (Diallo et al., 2020). For the WRC components with strong antifeedant effect on *G. f. fuscipes*, particularly geranyl acetone, messenger RNA (mRNA) transcripts of the predicted ORs were found to be affected in a mixed response including both up- and downregulation. Previously, Macharia et al. (2016) reported that chemosensory gene families in host selection are conserved across the sensilla of savannah (Morsitans) and riverine (Palpalis) groups of tsetse flies. We noted a reduced degree of repellency of blend K and WRC on *G. f. fuscipes* relative to the previously observed repellency for savannah tsetse flies (Bett et al., 2015; Olaide et al., 2019), consistent with previous findings for this tsetse fly species (Mbewe et al. (2019). Thus, the threshold of response to these compounds may vary between both groups of tsetse flies, dictated by their ecological adaptations (forest vs savannah areas). As such, the development of a repellent technology for control of tsetse flies must consider differences in formulations (e.g. dose and release rates) for populations in different ecology as previously highlighted by Tchouassi et al. (2019) for mosquitoes.

While both male and female tsetse flies are vectors of African trypanosomiasis (Sutherland and Tediosi, 2019; WHO, 2015), the repellent effect exhibited by both WRC and blend K varied by sex. Despite our general observation of reduced trap catches, significant differences were only evident for females for WRC and males for blend K (Fig 2; Table 1). Our result is consistent with previous findings for trap captures for the different sexes of *G. f. fuscipes* using the repellent WRC (Mbewe et al., 2019). Since trap captures of flies would consist of samples of both sexes at different ages (teneral, non-teneral, gravid), then if samples were dominated by a specific age compared to the other cohorts, this variable would be expected to differ between the treatments.

The three ketones evaluated are known repellents of savannah tsetse flies (Olaide et al., 2019; Torr et al., 1996). However, in the current study, acetophenone and 6-methyl-5-hepten-2-one had no repellent effect on *G. f. fuscipes* when tested individually, which may be associated with their higher vapour pressures relative to geranyl acetone. Our results showed that the estimated release rate of each of these two compounds was approximately three times higher than that of geranyl acetone (Table 2). It is known that behavioral responses of different insect species to odors are dose-and release rate-dependent (Antwi-Agyakwa et al., 2019; Jacob et al., 2018; Njihia et al., 2018; Nyasembe et al., 2015; Wondwosen et al., 2018). As such, although 6-methyl-5-hepten-2-one and geranyl acetone are components of natural human skin odor (Logan et al., 2008), the optimum repellent doses of these chemicals to *G. f. fuscipes* could be different from the dose found in the natural human odor. For instance, geranyl acetone alone, released at a higher dose compared to that found in the natural human odor, replicated the repellence of the whole human odor to savannah tsetse flies (Vale et al., 2012). Also, a different mode of formulation might be needed to test the repellent effects of acetophenone and 6-methyl-5-hepten-2-one, which would require additional research. Additionally, apart from spatial repellence, these compounds could also have antifeedant effect on *G. f. fuscipes*. Investigating these possibilities could provide an enhanced repellent blend of the compounds evaluated in this study for use in limiting human-tsetse contacts and disease transmission.

Overall, the current study demonstrates the repellent effect of blend K comprising 6-methyl-5-hepten-2-one, acetophenone and geranyl acetone on field populations of *G. f. fuscipes,* a key vector of sleeping sickness. Both blend K and known WRC, and geranyl acetone can contribute to the tool kit for the management of not only savannah tsetse flies, but also the riverine tsetse fly species *G. f. fuscipes*. These repellents could be used in materials such as hand bands, clothing and necklaces for personal protection from tsetse fly bites, and in a 'push-pull' strategy as components in the integrated control and elimination efforts of sleeping sickness.

Ethics approval and consent to participate

Not Applicable

Consent for publication

Not Applicable

Availability of data and materials

All data supporting the conclusion of this study are included in this published article. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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CRediT author statement

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Akazue, P.I., Ebiloma, G.U., Ajibola, O., Isaac, C., Onyekwelu, K., Ezeh, C.O., Eze, A.A., 2019. Sustainable elimination (zero cases) of sleeping sickness: how far are we from achieving this goal? Pathogens 8, 135. https://doi.org/10.3390/pathogens8030135.

Antwi-Agyakwa, A.K., Fombong, A.T., Deletre, E., Ekesi, S., Yusuf, A.A., Pirk, C., Torto, B., 2019. Lemon Terpenes Influence Behavior of the African Citrus Triozid *Trioza erytreae*

(Hemiptera: Triozidae). J. Chem. Ecol. 45, 934-945. https://doi.org/10.1007/s10886-019-01123-y.

Alsan, M., 2015. The effect of the tsetse fly on African development. Am. Econ. Rev. 105, 382-410. https://doi.org/10.1257/aer.20130604.

Beadell, J.S., Hyseni, C., Abila, P.P., Azabo, R., Enyaru, J.C.K., Ouma, J.O., Mohammed, Y.O., Okedi, L.M., Aksoy, S., Caccone, A., 2010. Phylogeography and Population Structure of *Glossina fuscipes fuscipes* in Uganda: Implications for Control of Tsetse. PLoS. Negl. Trop. Dis. 4: e636. https://doi.org/10.1371/journal.pntd.0000636.

Bett, M.K., Saini, R.K., Hassanali, A., 2015. Repellency of tsetse-refractory waterbuck (Kobus defassa) body to *Glossina pallidipes* (Diptera: Glossinidae): assessment of relative contribution of different classes and individual constituents. Acta Trop. 146, 17-24. https://doi.org/10.1016/j.actatropica.2015.02.017.

Bouyer, J., Carter, N.H., Batavia, C., Nelson, M.P., 2019. The ethics of eliminating harmful species: The case of the tsetse fly. BioScience 69, 125-135. https://doi.org/10.1093/biosci/biy155.

Bouyer, J., Stachurski, F., Gouro, A.S., Lancelot, R., 2009. Control of bovine trypanosomiasis by restricted application of insecticides to cattle using footbaths. Vet. Parasitol. 161, 187-193. https://doi.org/10.1016/j.vetpar.2009.01.018.

Büscher, P., Bart, J.M., Boelaert, M., Bucheton, B., Cecchi, G., Chitnis, N., Courtin, D., Figueiredo, L.M., Franco, J.R., Grébaut, P., Hasker, E., 2018. Do cryptic reservoirs threaten gambiense-sleeping sickness elimination? Trends Parasitol. 34, 197-207. https://doi.org/10.1016/j.pt.2017.11.008.

Capewell, P., Atkins, K., Weir, W., Jamonneau, V., Camara, M., Clucas, C., Swar, N.R., Ngoyi, D.M., Rotureau, B., Garside, P., Galvani, A.P., 2019. Resolving the apparent transmission paradox of African sleeping sickness. PLoS Biol. 17, e3000105. https://doi.org/10.1371/journal.pbio.3000105.

Chahda, J.S., Soni, N., Sun, J.S., Ebrahim, S.A.M., Weiss, B.L., Carlson, J.R., 2019. The molecular and cellular basis of olfactory response to tsetse fly attractants. PLoS Genet. 15, e1008005. https://doi.org/10.1371/journal.pgen.1008005.

Challier, A., Laveissiere, C., 1973. A new trap for catching Glossina: description and field trials. Cah ORSTOM Med. Entomol. Parasitol. 11, 251-262.

Courtin, F., Camara, M., Rayaisse, J.B., Kagbadouno, M., Dama, E., Camara, O., Traoré, I.S., Rouamba, J., Peylhard, M., Somda, M.B., Leno, M., 2015. Reducing human-tsetse contact significantly enhances the efficacy of sleeping sickness active screening campaigns: a promising result in the context of elimination. PLoS Negl. Trop. Dis. 9, e0003727. https://doi.org/10.1371/journal.pntd.0003727.

Diallo, S., Shahbaaz, M., Torto, B., Christoffels, A., Masiga, D., Getahun M.N., 2020. Cellular and molecular targets of waterbuck repellent blend odors in *Glossina fuscipes fuscipes* Newstead, 1910. Front. Cell. Neurosci. 14, 137. https://doi.org/10.3389/fncel.2020.00137.

Esterhuizen, J., Rayaisse, J.B., Tirados, I., Mpiana, S., Solano, P., Vale, G.A., Lehane, M.J., Torr, S.J., 2011. Improving the cost-effectiveness of visual devices for the control of riverine tsetse flies, the major vectors of human African trypanosomiasis. PLoS Negl. Trop. Dis. 5, e1257. https://doi.org/10.1371/journal.pntd.0001257.

FAO, 1992a. Training manual for tsetse control personnel Volume 1. Tsetse biology, systematics and distribution; techniques. Pollock, J.N, (Ed.), Food and Agricultural Organization of the United Nations, Rome, pp. 63-90.

FAO, 1992b. Training manual for tsetse control personnel Volume 4. Use of attractive devices for tsetse survey and control. Hursey, B.S., Slingenbergh, J.H.W., (Eds.), Food and Agricultural Organization of the United Nations, Rome, pp. 68-94.

Ghaninia, M., Ignell, R., Hansson, B.S., 2007. Functional classification and central nervous projections of olfactory receptor neurons housed in antennal trichoid sensilla of female yellow fever mosquitoes, *Aedes aegypti*. Eur. J. Neurosci. 26, 1611-1623. https://doi.org/10.1111/j.1460-9568.2007.05786.x.

Gloria-Soria, A., Dunn, W.A., Yu, X., Vigneron, A., Lee, K.Y., Li, M., Weiss, B.L., Zhao, H., Aksoy, S., Caccone, A., 2018. Uncovering Genomic Regions Associated with Trypanosoma Infections in Wild Populations of the Tsetse Fly *Glossina fuscipes*. G3: Genes, Genomes, Genet. 8, 887-897. https://doi.org/10.1534/g3.117.300493.

Hargrove, J.W., Williams, B.G., 1995. A cost-benefit analysis of feeding in female tsetse. Med. Vet. Entomol. 9, 109-119. https://doi.org/10.1111/j.1365-2915.1995.tb00166.x.

Holmes, P., 2014. First WHO meeting of stakeholders on elimination of gambiense human African trypanosomiasis. PLoS Negl. Trop. Dis. 8, e3244. https://doi.org/10.1371/journal.pntd.0003244.

Jacob, J.W., Tchouassi, D.P., Lagat, Z.O., Mathenge, E.M., Mweresa, C.K., Torto, B., 2018. Independent and interactive effect of plant-and mammalian-based odors on the response of the malaria vector, *Anopheles gambiae*. Acta Trop. 185, 98-106. https://doi.org/10.1016/j.actatropica.2018.04.027.

Lindh, J.M., Torr, S.J., Vale, G.A., Lehane, M.J., 2009. Improving the cost-effectiveness of artificial visual baits for controlling the tsetse fly *Glossina fuscipes fuscipes*. PLoS Negl. Trop. Dis. 3, e474. https://doi.org/10.1371/journal.pntd.0000474.

Logan, J.G., Birkett, M.A., Clark, S.J., Powers, S., Seal, N.J., Wadhams, L.J., Mordue, A.J., Pickett, J.A., 2008. Identification of human-derived volatile chemicals that interfere with attraction of *Aedes aegypti* mosquitoes. J. Chem. Ecol. 34, 308. https://doi.org/10.1007/s10886-008-9436-0.

Macharia, R., Mireji, P., Murungi, E., Murilla, G., Christoffels, A., Aksoy, S., Masiga, D., 2016. Genome-wide comparative analysis of chemosensory gene families in five tsetse fly species. PLoS Negl. Trop. Dis. 10, e0004421. https://doi.org/doi:10.1371/journal.pntd.0004421.

Mahamat, M.H., Peka, M., Rayaisse, J.B., Rock, K.S., Toko, M.A., Darnas, J., Brahim, G.M., Alkatib, A.B., Yoni, W., Tirados, I., Courtin, F., 2017. Adding tsetse control to medical activities contributes to decreasing transmission of sleeping sickness in the Mandoul focus (Chad). PLoS Negl. Trop. Dis. 11, e0005792. https://doi.org/10.1371/journal.pntd.0005792.

Manangwa, O., Nkwengulila, G., Ouma, J.O., Mramba, F., Malele, I., Dion, K., Sistrom, M., Khan, F., Aksoy, S., Caccone, A., 2017. Genetic diversity of *Glossina fuscipes fuscipes* along the shores of Lake Victoria in Tanzania and Kenya: implications for management. Parasit. Vectors 10, 268. https://doi.org/10.1186/s13071-017-2201-x.

Mbewe, N.J., Saini, R.K., Irungu, J., Yusuf, A.A., Pirk, C.W.W., Torto, B., 2019. Responses of *Glossina fuscipes fuscipes* to visually attractive stationary devices baited with 4-

methylguaiacol and certain repellent compounds in waterbuck. PLoS Negl. Trop. Dis. 13, e0007510. https://doi.org/10.1371/journal.pntd.0007510.

Mbewe, N.J., Saini, R.K., Torto, B., Irungu, J., Yusuf, A.A., Pirk, C.W.W., 2018. Sticky small target: an effective sampling tool for tsetse fly *Glossina fuscipes fuscipes* Newstead, 1910. Parasit. Vectors 11, 268. https://doi.org/10.1186/s13071-018-2840-6.

Njihia, T.N., Torto, B., Murungi, L.K., Irungu, J., Mwenda, D.M., Babin, R., 2018. Ripe coffee berry volatiles repel second instar nymphs of Antestia bugs (Heteroptera: Pentatomidae: *Antestiopsis thunbergii*). Chemoecology 28, 91-100. https://doi.org/10.1007/s00049-018-0259-3.

Nyasembe, V.O., Tchouassi, D.P., Mbogo, C.M., Sole, C.L., Pirk, C., Torto, B., 2015. Linalool oxide: generalist plant based lure for mosquito disease vectors. Parasit. Vectors 8, 581. https://doi.org/10.1186/s13071-015-1184-8.

Olaide, O.Y., Tchouassi, D.P., Yusuf, A.A., Pirk, C.W.W., Masiga, D.K., Saini, R.K., Torto, B., 2019. Zebra skin odor repels the savannah tsetse fly, *Glossina pallidipes* (Diptera: Glossinidae). PLoS Negl. Trop. Dis. 13, e0007460. https://doi.org/10.1371/journal.pntd.0007460.

Oloo, F., Sciarretta, A., Mohamed-Ahmed, M.M., Kröber, T., McMullin, A., Mihok, S., Guerin, P.M., 2014. Standardizing visual control devices for tsetse flies: East African species *Glossina fuscipes fuscipes* and *Glossina tachinoides*. PLoS Negl. Trop. Dis. 8, e3334. https://doi.org/10.1371/journal.pntd.0003334.

Omolo, M.O., Hassanali, A., Mpiana, S., Esterhuizen, J., Lindh, J., Lehane, M.J., Solano, P., Rayaisse, J.B., Vale, G.A., Torr, S.J., Tirados, I., 2009. Prospects for developing baits to control *Glossina fuscipes* spp., the major vector of human African trypanosomiasis. PLoS Negl. Trop. Dis. 3, e435. https://doi.org/10.1371/journal.pntd.0000435.

Opiro, R., Saarman, N.P., Echodu, R., Opiyo, E.A., Dion, K., Halyard, A., Dunn, A.W., Aksoy, S., Caccone, A., 2017. Genetic diversity and population structure of the tsetse fly *Glossina fuscipes fuscipes* (Diptera: Glossinidae) in Northern Uganda: Implications for vector control. PLoS. Negl. Trop. Dis. 11: e0005485. https://doi.org/10.1371/journal.pntd.0005485.

Otter, C.D., Tchicaya, T., Schutte, A.M., 1991. Effects of age, sex and hunger on the antennal olfactory sensitivity of tsetse flies. Physiol. Entomol. 16, 173-182. https://doi.org/10.1111/j.1365-3032.1991.tb00554.x.

Ouedraogo, L., den Otter, C.J., 2018. Comparison of single cell sensitivities to acetone, 1-octen-3-ol and 3-methylphenol in the riverine tsetse species *Glossina fuscipes fuscipes* and *G. palpalis palpalis*. J. Insect Physiol. 107, 144-51. https://doi.org/10.1016/j.jinsphys.2018.03.003.

Rayaisse, J.B., Esterhuizen, J., Tirados, I., Kaba, D., Salou, E., Diarrassouba, A., Vale, G.A., Lehane, M.J., Torr, S.J., Solano, P., 2011. Towards an optimal design of target for tsetse control: comparisons of novel targets for the control of Palpalis group tsetse in West Africa. PLoS Negl. Trop. Dis. 5, e1332. https://doi.org/10.1371/journal.pntd.0001332.

Rock, K.S., Torr, S.J., Lumbala, C., Keeling, M.J., 2017. Predicting the impact of intervention strategies for sleeping sickness in two high-endemicity health zones of the Democratic Republic of Congo. PLoS Negl. Trop. Dis. 11, e0005162. https://doi.org/10.1371/journal.pntd.0005162.

Saini, R.K., Orindi, B.O., Mbahin, N., Andoke, J.A., Muasa, P.N., Mbuvi, D.M., Muya, C.M., Pickett, J.A., Borgemeister, C.W., 2017. Protecting cows in small holder farms in East Africa from tsetse flies by mimicking the odor profile of a non-host bovid. PLoS Negl. Trop. Dis. 11, e0005977. https://doi.org/10.1371/journal.pntd.0005977.

Simarro, P.P., Jannin, J., Cattand, P., 2008. Eliminating human African trypanosomiasis: where do we stand and what comes next? PLoS Med. 5, e55. https://doi.org/10.1371/journal.pmed.0050055.

Simo, G., Rayaisse, J.B., 2015. Challenges facing the elimination of sleeping sickness in west and central Africa: sustainable control of animal trypanosomiasis as an indispensable approach to achieve the goal. Parasit. Vectors 8, 640. https://doi.org/10.1186/s13071-015-1254-y.

Sudarshi, D., Lawrence, S., Pickrell, W.O., Eligar, V., Walters, R., Quaderi, S., Walker, A., Capewell, P., Clucas, C., Vincent, A., Checchi, F., 2014. Human African trypanosomiasis presenting at least 29 years after infection - what can this teach us about the pathogenesis and control of this neglected tropical disease? PLoS Negl. Trop. Dis. 8, e3349. https://doi.org/10.1371/journal.pntd.0003349.

Sutherland, C.S., Stone, C.M., Steinmann, P., Tanner, M., Tediosi, F., 2017. Seeing beyond 2020: an economic evaluation of contemporary and emerging strategies for elimination of *Trypanosoma brucei gambiense*. The Lancet Global Health 5, e69-79. https://doi.org/10.1016/S2214-109X(16)30237-6.

Sutherland, C.S., Tediosi, F., 2019. Is the elimination of 'sleeping sickness' affordable? Who will pay the price? Assessing the financial burden for the elimination of human African trypanosomiasis *Trypanosoma brucei gambiense* in sub-Saharan Africa. BMJ Global Health 4, e001173. https://doi.org/10.1136/bmjgh-2018-001173.

Tchouassi, D.P., Jacob, J.W., Ogola, E.O., Sang, R., Torto, B., 2019. Aedes vector–host olfactory interactions in sylvatic and domestic dengue transmission environments. Proc. R. Soc B 286, 20192136. https://doi.org/10.1098/rspb.2019.2136.

Tirados, I., Esterhuizen, J., Kovacic, V., Mangwiro, T.C., Vale, G.A., Hastings, I., Solano, P., Lehane, M.J., Torr, S.J., 2015. Tsetse control and Gambian sleeping sickness; implications for control strategy. PLoS Negl. Trop. Dis. 9, e0003822. https://doi.org/10.1371/journal.pntd.0003822.

Torr, S.J., Mangwiro, T.N., Hall, D.R., 1996. Responses of *Glossina pallidipes* (Diptera: Glossinidae) to synthetic repellents in the field. Bull. Entomol. Res. 86, 609-616. https://doi.org/10.1017/S0007485300039419.

Torr, S.J., Vale, G.A., 2015. Know your foe: lessons from the analysis of tsetse fly behavior. Trends Parasitol. 31, 95-99. https://doi.org/10.1016/j.pt.2014.12.010.

Vale, G.A., Hall, D.R., Chamisa, A., Torr, S.J., 2012. Towards an Early Warning System for Rhodesian Sleeping Sickness in Savannah Areas: Man-Like Traps for Tsetse Flies. PLoS Negl. Trop. Dis. 6, e1978. https://doi.org/10.1371/journal.pntd.0001978.

Vale, G.A., Hargrove, J.W., Solano, P., Courtin, F., Rayaisse, J.B., Lehane, M.J., Esterhuizen, J., Tirados, I., Torr, S.J., 2014. Explaining the host-finding behavior of blood-sucking insects: computerised simulation of the effects of habitat geometry on tsetse fly movement. PLoS Negl. Trop. Dis. 8, e2901. https://doi.org/10.1371/journal.pntd.0002901.

Venables, W.N., Ripley, B.D., 2002. Modern applied statistics with S, fourth ed. Springer, New York.

Vieira, F.G., Sánchez-Gracia, A., Rozas. J., 2007. Comparative genomic analysis of the odorant-binding protein family in 12 Drosophila genomes: purifying selection and birth-and-death evolution. Genome Biol. 8, R235. https://doi.org/10.1186/gb-2007-8-11-r235.

Wamwiri, F.N., Nkwengulila, G., Clausen, P.H., 2007. Hosts of *Glossina fuscipes fuscipes* and *G. pallidipes* in areas of western Kenya with endemic sleeping sickness, as determined using an egg-yolk (IgY) ELISA. Ann. Trop. Med. Parasitol. 101, 225-232. https://doi.org/10.1179/136485907X156979.

WHO, 2015. Report of the first WHO stakeholders meeting on rhodesiense human African trypanosomiasis. World Health Organization, Geneva, Switzerland, pp. 1-60.

Wondwosen, B., Birgersson, G., Tekie, H., Torto, B., Ignell, R., Hill, S.R., 2018. Sweet attraction: sugarcane pollen-associated volatiles attract gravid *Anopheles arabiensis*. Malar. J. 17, 90. https://doi.org/10.1186/s12936-018-2245-1.

Supplementary material

Table S1

Randomized 6 × 6 Latin square design. Treatments were moved to new sites daily according to the randomized Latin square design. Three 6 × 6 Latin square experiments ran concurrently, one on Chamaunga, Rusinga and Manga islands. In total, the 6 × 6 Latin square-designed experiment was replicated six times giving thirty-six data points for each treatment.

Site	**Treatment**					
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**
1	MH	AP	Blend K	GA	WRC	Trap alone
2	Blend K	MH	WRC	AP	Trap alone	GA
3	GA	Trap alone	AP	WRC	MH	Blend K
4	AP	GA	MH	Trap alone	Blend K	WRC
5	WRC	Blend K	Trap alone	MH	GA	AP
6	Trap alone	WRC	GA	Blend K	AP	MH