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Prospects for developing efficient targets for the xenomonitoring and control of *Simulium damnosum* s.l., the major vectors of onchocerciasis in Africa

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Abstract

Despite the ethical issues concerning the continued use of Human Landing Catches (HLC) to monitor the *Simulium damnosum* complex for epidemiological monitoring of onchocericasis, few attempts to develop alternatives have been reported. In studies on a wild population of *S. damnosum* in Burkina Faso, we tested visual targets (different sizes and shapes) and olfactory stimuli (CO_2, and POCA and BG-lure® odour blends) for their ability to attract and collect host seeking blackflies. At each trap, blackflies were caught with appropriately sized electrocuting grids and results from Latin square design tests were compared. Throughout, HLCs captured more blackflies than the targets. Of the traps tested, small targets (0.0625 and 0.5 m^2) were the most efficient visual lure in terms of the number of *S. damnosum* captured per unit area 1.7–5× more than larger targets. Overall, results suggested that sticky black targets of horizontal rectangular shape (0.125–0.5 m^2) and baited with a POCA and/or CO_2 mixture could provide a cheap practical field alternative to HLC for onchocerciasis xenomonitoring, subject to confirmation that the design has no inherent bias for certain members of the *S. damnosum* species complex.

KEYWORDS
onchocerciasis, simulium, trapping, surveillance and control

INTRODUCTION

Onchocerciasis, also known as 'river blindness', one the major cause of blindness globally, is still a public health problem. A recent estimate indicates that around 198 million people live in areas with high risk of the transmission of *Onchocerca volvulus* by *Simulium damnosum* s.l. (WHO, 2019). In 2015, the disease caused the loss of 1.3 million disability-adjusted life-years (DALYs) (WHO, 2021). Sub-Saharan African countries bear the heaviest burden and, despite over four decades of effort, the disease remains endemic in 31 countries of this continent. Over a period of 30 years, Onchocerciasis Control Program (OCP) and endemic countries successfully eliminated onchocerciasis as a public health problem in West Africa through vector control by larviciding and mass drug treatment with ivermectin of the human population (Thylefors & Alleman, 2006). As agreed during the WHO/NTD road map meeting in January 2021, WHO and endemic countries plan to eliminate transmission of river blindness in at least 12 countries by 2030 using mass drug administration (MDA) with ivermectin (WHO, 2021). Vector control implementation with larvicides is too costly and not feasible in many places due to inaccessibility or abundance of rivers and larval habitats (WHO, 2015).

WHO describes a three-phase process for the verification of elimination of onchocerciasis transmission: the MDA intervention phase, the post-treatment surveillance phase, and the post-elimination surveillance phase (WHO, 2016). To move from one phase to the following and for post-elimination surveillance, WHO (2016) recommend xenomonitoring of the disease, which is defined as the assessment of the prevalence of *O. volvulus* infective larvae in blackfly vectors, biting rates, and other entomological indices measured during surveillance (Otabil et al., 2018). WHO recommends collecting a minimum of 6000 female blackflies in each transmission zone to accurately predict the prevalence of the parasite. Human landing capture (HLC), whereby human volunteers capture blackflies that land on them for a blood-meal, is the gold standard for the collection of adult female *S. damnosum* s.l (Otabil et al., 2018). Although this method has given excellent results, in the past, it poses ethical and/or economic problems by exposing an individual to potentially infectious bites (Jacobi et al., 2010). Accordingly, in 2016 and in the context of onchocerciasis elimination, WHO stated that 'more investigations should be carried out to define appropriate and standardized protocols for fly catching' (WHO, 2016). Therefore, there is an urgent need to develop standard tools that can routinely collect such large numbers of flies, without the need of expose volunteers to potentially infective bite.

Several traps have been developed with valuable level of success, either for onchocerciasis surveillance or vector control with variable levels of success, of which Bellec traps and silhouette traps are among the best known (Service, 1977). Recently, the Esperanza Window Trap (EWT) was developed for the xenomonitoring of onchocerciasis (Rodríguez-Pérez et al., 2013; Toé et al., 2014). This trap gave very encouraging results and the possibility to be deployed by local communities (Loum et al., 2017). However, the authors pointed out that the EWT needs to be optimized with further research on the effect of visual and/or olfactory cues (Otabil et al., 2018). They highlighted the absence of key knowledge about *S. damnosum* complex' host seeking behaviour, crucial for the further improvement of the traps. It is still unknown how blackflies respond to simple visual stimuli such as variations in size, shape, or colour of the host, or how blackflies respond to certain host odours.

Behavioural studies on visual responses to target shapes have been carried out with other hematophagous insects and have proven immensely successful with tsetse, leading to the development of effective targets for both trypanosomiasis surveillance and vector control (Tirados et al., 2015; Vander Kelen et al., 2020). Conversely, few studies conducted on developing targets for either monitoring or controlling the vectors of onchocerciasis have been reported. Though it is generally recognized that visual cues provide important species information on prospective host identity, there are few studies on *S. damnosum s*.l. blackflies responses to visual stimuli. Yet, targets are of great interest for surveillance because they are relatively cheap and easy to deploy by the communities themselves. Moreover, and as for tsetse flies, such targets could be impregnated with insecticides to which *S. damnosum* complex members are sensitive and thus represent an alternative to the use of larviciding for vector control.

The present study was conducted with the aim of identifying the responses of *S. damnosum* s.l. to several visual and/or olfactory stimuli, as the first step towards the aim of developing catching/killing devices effective for xenomonitoring and/or control of onchocerciasis vectors in Africa.

METHODS

Study sites

This study was conducted in Burkina Faso near the villages of Bodadiougou in the Cascades region, Bapla in the South-West region, and Samandeni in the Hauts-Bassins region. The primary criterion for the selection of these sites was the permanent presence of *S. damnosum* s.l. breeding sites. The Bougouriba River flows through the Bapla site. This has been an endemic watercourse for onchocerciasis since the period of time of the OCP (Koala et al., 2017). A small dam provides permanent blackfly breeding sites. The same is true for the village of Bodadiougou, where the Comoé River flows. In Bodadiougou, breeding sites are maintained by the 'Comoé Dam' located upstream of the river. Finally, the Mouhoun river crosses the village of Samandeni, where the 'Samandeni dam' creates and maintains large breeding sites for *S. damnosum* complex. Onchocerciasis is still endemic in the villages surrounding the study sites, particularly around Bodadiougou. Indeed, a recrudescence of onchocerciasis was recently reported from Bodadiougou village with high prevalence and infectivity rates (Koala et al., 2017, 2019). In all breeding sites nearby, both sibling savanna species of the *S. damnosum* complex are present, namely *Simulium damnosum* s.s. and *Simulium sirbanum*. Other arthropod vectors, such as Glossina species that transmit trypanosomiasis, have been identified along the Comoé river in Burkina Faso (Rayaisse et al., 2011, Tirados et al., 2011). *G. palpalis gambiensis* and *G. tachinoides* are active in the riverine forest during the dry season and therefore share the same environment with *S. damnosum* s.l. in Bodadiougou village.

Lures and collecting devices

To evaluate the responses of blackflies to different visual and olfactory cues, we used electric grids which have been used to develop targets against tsetse flies (Rayaisse et al., 2010; Tirados et al. 2011; Vale, 2015). The electric grids consisted of multiple 0.2 mm diameter fine copper wires, spaced 4 mm apart, and mounted in aluminium frames. Black felt is placed behind the wires. Grids are electrified by batteries (12 V, 45 Ah) connected to high-voltage transformers. The grids were mounted on grey metal collection trays ~5 cm deep, containing soapy water to retain and collect the electrocuted flies.

We used two types of electric grids for our study, referred to as the Etarget and the Flanking net, or Fnet (Figure 1). The Etarget, consisted of a black cotton fabric panel sandwiched between two electric grids. The fabric serves as the visual attractant based on its colour,

ARTIFICIAL BAITS FOR THE COLLECTION OF *SIMULIUM DAMNOSUM S.*L

Medical and Veterinary
Entomology

Royal
Entomological
Society

237

FIGURE 1 Electric grids catching devices with Etarget (grids with black cloth) at the right and flanking net (Fnet) at the right

shape or size, and the electric grids' wires allowed to electrocute the flies that were attracted by the target, and which flew to land on it. Thus, the Etarget target attracted flies and the grids killed them as they collided with the wires. In this study, we used several Etarget designs depending on the purpose of each experiment.

Fnets consisted of a simple electric grid with electrified wires and were assumed to be invisible to Simulium spp. Fnets were used in combination with Etargets to passively collect 'circling' blackflies. Indeed, of the *S. damnosum* s.l. attracted to the targets, not all will land, and many left the targets, after circling the putative host without landing on them. As with tsetse flies, we assumed that the Fnets were 'invisible' to *S. damnosum* complex. Therefore, we assumed that flies killed by the Fnets were so as they 'collided accidentally' with it, rather than as the results of a 'deliberate landing response'. To estimate the proportion of blackflies circling the target, F-nets and Etarget were placed side by side. The Etarget and the F-net had separate collection bins to allow independent quantification of flies collected by each device. The Fnet had a standard size (1.0 m height × 0.5 m wide) for all experiments in which they were used.

In experiments with artificial odour baits, the electric grids were replaced with clear adhesive plastic sheeting (Barrettine Environmental Health, UK). This adhesive sheet was wrapped around the surface of the different targets to catch black flies and other hematophagous insects.

Experimental design

Previous studies on circardian rhythms showed bimodal patterns in the daily activity peaks of female blackflies (LeBerre, 1966;

Philipon, 1977). The first peak occurs in the morning between 8 and 10 am and the second peak, which is the most important with a higher number of flies, occurs between 15.00 and 19.00 h. All experiments were therefore conducted daily between 14.00 and 18.00 h, when *S. damnosum* s.l. are more active. The experiments were conducted following Latin-square designs (# days × #sites x #treatments) to balance position effects on the target yields.

Series A: Assessment of blackfly response to Etargets of different sizes

Experiment 1

These experiments aimed to assess responses of *S. damnosum* s.l. to targets of different sizes. To do so, first we compared the catches obtained with three different sizes of black targets: a small Etarget (0.25 m × 0.25 m either 0.0625 m^2), a medium Etarget (0.5 m × 0.5 m; 0.25 m^2) and a large Etarget (1.0 m × 1.0 m; 1 m^2). The experiment was carried out for nine consecutive days in Bodadiougou breeding site.

Experiment 2

In a Latin square design, we added a flanking net to each of the three Etargets in experiment 1 and a collection team that performed HLC simultaneously. This experiment was conducted for 12 consecutive days at Bodadiougou breeding site and replicated at the Bapla breeding site.

Series B: Assessment of blackfly response to Etargets of different shapes

Experiment 3 and 4

This experiment aimed to assess the differential response of vectors to rectangles (oriented vertically or horizontally) and squares. We compared Etargets of three different shapes: a vertical rectangle (1.0 m height × 0.5 m wide), a horizontal rectangle (0.5 m height × 1.0 m wide), and a square (0.7 m height × 0.7 m wide). All Etargets had the same surface area of 0.5 m^2. The experiment was repeated with (Experiment 4) and without Fnets (Experiment 3). A HLC team also collected blackflies during experiment 3.

Series C: Assessment of combining shape and size

Experiment 5 and Experiment 6

Based on the results of the two previous experiments, we assessed the response of blackflies to horizontal targets (the most efficient shape from series 2) of decreasing size. The objective of this experiment was to assess the size of horizontal targets that is the most efficient at catching female blackflies. Three horizontal targets were designed as follows: small target (0.25 m wide × 0.13 m high; 0.0325 m^2), medium Etarget (0.5 m wide × 0.25 m high; 0.12 5m^2) and a large horizontal Etarget (1.0 wide × 0.5 m high; 0.5 m^2). The three designs were compared with (Experiment 5) and without (Experiment 6) an Fnet in Bapla and Bodadiougou breeding sites. Experiments were conducted for nine consecutive days in those sites.

Series D: Assessment of blackfly responses to different artificial odour baits

Experiment 7. Blackfly response to targets baited with carbon dioxide (CO$_2$)

This Latin square aimed to assess responses of blackflies to carbon dioxide and consisted of four treatments all using a black horizontal target (1.0 wide × 0.5 m high; 0.5 m^2): an unbaited target as control, a target baited with CO$_2$, an unbaited target flanked by an Fnet, and a target baited with CO$_2$ and flanked by an Fnet. CO$_2$ was produced using a yeast and sugar generation system as previously described (Guerenstein et al., 1995; Smallegange et al., 2010). The experiment was conducted for 12 days in Bodadiougou breeding sites.

Experiment 8. Blackfly response to targets baited with artificial odours

In this experiment, we compared the attractiveness of several artificial odours associated with sticky large horizontal targets (1.0 m wide × 0.5 m high). Odour experiments were conducted with different odours baits. The first odour was a blend hereinafter referred to as POCA, containing: 3-n-propylphenol (released at 0.01 mg/h; 'P'), 1-octen-3-ol (or octenol, released at 0.1 mg/h; 'O'), 4-methylphenol (or p-cresol, released at 0.4 mg/h; 'C') and acetone ('A'). These odours were dispensed through plastic sachets of 0.15 mm thick was filled with 4 ml of 4-methylphenol, 3-n-propylphenol, and octenol in proportion 8:1:4 as described previously by Torr et al. (1997) and accompanied with 2 ml acetone in a bottle. The second odour was the commercial human scent lure BG-lure® which is a blend of lactic acid, ammonia, hexanoic acid, and octenol (Arimoto et al., 2015), and the third odour, the CO$_2$ which was produced as previously described. Six treatments were made as follows: an unbaited simple target without odours (control), a target with CO$_2$, a target baited with BG-lure, a target baited with POCA blend alone, a target baited with BG-lure and CO$_2$, and a target baited with POCA and CO$_2$. The experiment was conducted for 12 days in Bapla breeding sites (2 full replicates of the Latin square).

Processing of samples

Diptera of medical or veterinary importance obtained during the experiments were collected, counted, and preserved in 80% ethanol. In addition to Simuliidae and Glossinidae, they included Tabanidae and Muscidae. Samples were identified morphologically using a binocular dissecting microscope. Specimen of *S. damnosum* complex were distinguished from other Simulium species based on criteria described in Davies and Crosskey (1992). The savanna cytospecies (*S. damnosum* [s.s.] and *S. sirbanum*) were distinguished from other members of *S. damnosum* complex based on the coloration of the wing tufts, procoxa, antennae, and scutal tufts (Davies & Crosskey, 1992; Wilson et al., 1993). Newly emerged *S. damnosum* s.l. or 'neonates', were identified based on the incomplete pigmentation of their legs (Crosskey, 1990; Philipon, 1977). Ovipositing blackflies were identified based on the presence of eggs in the ovaries.

Data analysis

The primary outcome metrics were (1) the number of flies captured per day and per site (also called the 'catch'), and (2) the number of flies captured per area unit (m^2) of the target (also called the 'catch density'), and (3) the proportion of flies that effectively landed on the target (called 'landing response'), compared to the total number of flies collected (i.e., Etarget + Fnet).

Catches

The daily number of flies caught is the most important factor. For the analyses, the catch was considered as the number of *S. damnosum* s.l. caught by the Etarget, or the number of flies caught by one Etarget and Fnet when Fnet was included in the design.

Catch density

In the context of vector control, when many targets need to be deployed simultaneously, the area of the target becomes more important, since larger traps would cost more to produce, transport, store, and deploy. We calculated the catch density of each target by dividing the daily catches by the target's area. Note that in experiments where Etargets were accompanied with Fnets, we do not include the area of flanking net in the catch density calculation. For the statistical analyses of catches and catch density, the data were analysed in R version 3.5.2 with the packages MASS (Venables and Ripley, 2002) and multcomp (Hothorn et al., 2008). The daily fly collection was analysed with Generalized Linear Models (GLM) using a Poisson distribution and negative binomial distribution, the latter giving a better fit according to residual deviance and Akaike Information Criteria. The significance of changes in deviance was assessed by chi-squared. Post-hoc Tukey contrasts using the 'emmeans' package to allow comparisons between trapping methods (Russell et al., 2019). For all analyses, the level of significance was established at $p < 0.05$.

Landing response (LR)

The flight and landing behaviour of simuliids towards their hosts is not well understood. Previous observations indicated that a proportion of the simuliids attracted to a host, some hover around the host without landing on it (Crosskey, 1990). Insights into such phenomenon is crucial in the development of artificial targets that exploit the natural host-seeking behaviour. The association of invisible flanking nets provides a better understanding of their flight and landing behaviour because it allowed us to estimate the landing response. The landing response of an Etarget was calculated as the proportion of flies collected by an Etarget relative to the total number of flies. We explored whether the landing response was a function of the size or the shape of the targets, or it varied for the different olfactory baits. Landing responses were compared using Logistic regressions, being the catch from the Etarget specified as the y-variable and the total catch (Etarget+ Fnet) as the binomial denominator. Days, sites, and treatments (e.g., shape, size, odours) were specified as factors. Statistically significant differences were assessed comparing the full model with the model without the treatment factor. The significance of changes in deviance was assessed by chi-squared or, if the data were overdispersed, an *F*-test following re-scaling. For all analyses, the level of significance was established at $p < 0.05$.

Ethics statement

We obtained the approval of the Institutional Ethical Committee for Research in Health Sciences of Burkina Faso (approval No A028-2018/CEIRES) and the institutional Ethical Committee at the Liverpool School of Tropical Medicine (Research Protocol 19-014/LSTM REC). All the people involved in the capture of blood-sucking insects by the traps or the HLC signed consent forms after being informed of the project's activities. Consent forms were in French and in their local languages (Dioula). People involved in the HLC were trained in the capture of blackflies and were able to reduce high exposure to transmission by reducing the time of collection after landing. To reduce the risk further, all the local participants in the study received ivermectin treatment as part of the mass distribution campaigns from the national programme.

RESULTS

Diversity of vectors collected

During the experiments, the traps collected several taxa of diptera with medical interest. Among them, we collected mainly simuliids, tsetse, horseflies, deerflies, and stable flies. Other collected insects included domestic flies, dragonflies, butterflies, and several hemipterans. On average, the proportion of *S. damnosum* s.l. varied according to the traps and the days of collection. In terms of the physiological stages of *S. damnosum* s.l., the traps caught host-seeking simuliids, neonate simuliids, ovipositing females, and males. The proportion of each stage varied according to the day, the site, and the position of the trap. Newly emerged flies, ovipositing gravid flies and male simuliids were caught to a much greater extent when traps were placed close to rapids (2–4 m) in sites where the riverbed was level with the banks (Bapla site). Host-seeking simuliids were mostly captured when the traps were exposed several metres from the rapids (10–20 m) and most often in embanked rivers such as Bodadiougou or Samandeni. For the statistical analyses, we considered all physiological categories of *S. damnosum* s.l. as belonging to one group and the analyses were done in this sense.

The effect of target size (experiments series A)

In the experiment 1 conducted at Bodadiougou, results showed that target size had a significant effect on catches with the large target (mean catch = 10, 95% CI: [3, 17] $p < 0.028$) collecting significantly more *S. damnosum* s.l. per day than the medium target (mean catch = 4, 95% CI: [1, 7] $p < 0.028$) and small target (mean catch = 1, 95% CI: [0, 1] $p < 0.001$) (Figure 2). The medium target caught significantly more *S. damnosum* s.l. per day than the small target ($p < 0.016$). Day and location had no significant effect on catch. When comparing the catch density, the small target caught the highest number of *S. damnosum* s.l. per area but there were no statistically significant differences between catch densities. The large (mean catch = 6, 95% CI: [3, 9]) and medium (mean catch = 8, 95% CI: [4, 12]) targets collected significantly more *G. palpalis*/day than the small target (mean catch = 1, 95% CI: [0, 1], $p < 1e-04^{***}$). The number of *G. tachinoides* was too low for statistical comparisons.

In experiment 2, the same targets with accompanied Fnet were compared to the HLC in a subsequent experiment at Bodadiougou breeding site and repeated at Bapla breeding site. At Bodadiougou,
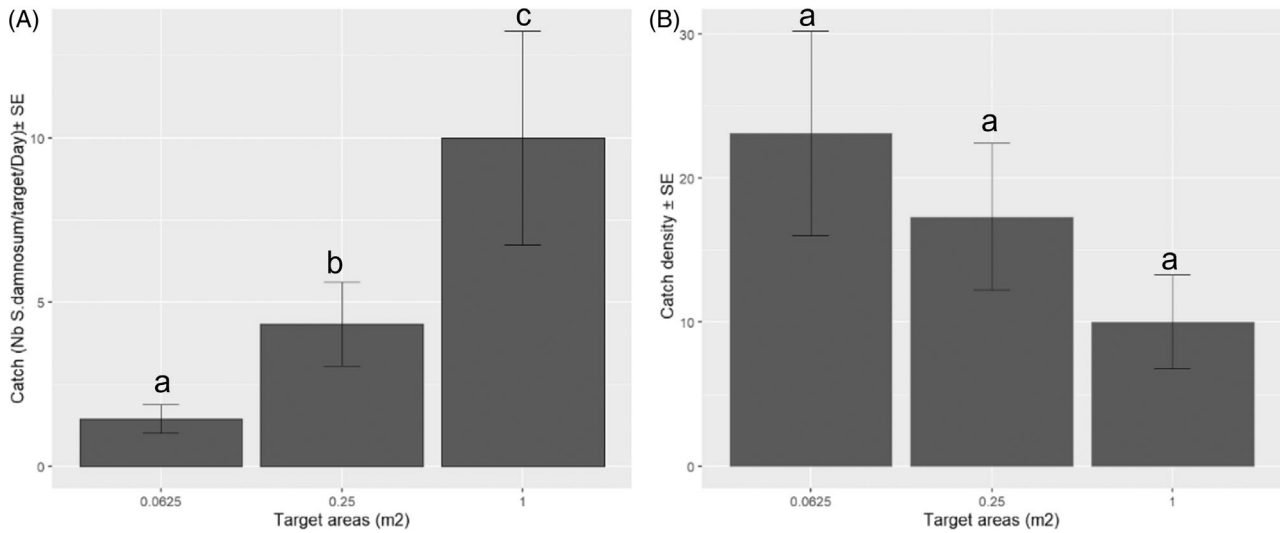
FIGURE 2 Comparison of decreasing size of Etarget at Bodadiougou in experiment 1. (a) Mean daily catch of *S. damnosum* caught by the decreasing size. (b) Comparison of the catch densities of the three targets. Different letters denote significant differences at $p < 0.01$ by negative binomial generalized linear models
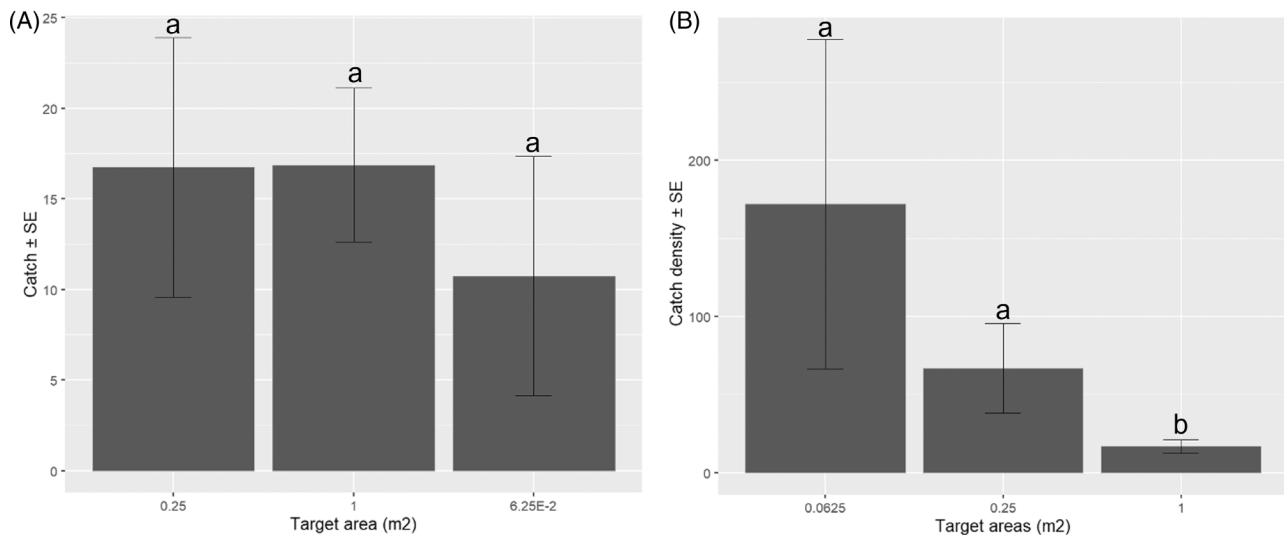

FIGURE 3 Comparison of decreasing size of Etargets and accompanied Fnet at Bodadiougou in experiment 2. (a) Mean daily catch of S. damnosum caught by the decreasing size of targets. (b) Comparison of the catch densities of the three targets. Different letters denote significant differences at $p < 0.01$ by negative binomial generalized linear models

HLC (mean catch = 298, 95% CI: [216, 380]) collected significantly more *S. damnosum* s.l/day than each of the targets ($p < 0.001$) but there was no significant difference between the catches of different Etargets. Target size influenced the catch density ($p < 0.00232$) with small target + Fnet (mean catch = 172, 95% CI: [0, 249]) catch significantly higher than that of the large target + Fnet (mean catch = 17, 95% CI: [7, 27], $p < 0.00188$). No significant differences were observed between the small and medium size treatments (mean catch = 67, 95% CI: [0, 135], $p < 0.058$). No difference between the catch density of the medium and the large target. Study day and target's locations had a small effect on targets yields (Figure 3b). The target size significantly influenced the percentage of *S. damnosum* s.l. landing effectively on the Etarget ($F_{[2]} = 6.84$,

$p < 0.005$) with LR varying from 25% for the small to 57% for the large Etarget. No statistical differences were observed between the number of *G. palpalis* collected by the different targets or for the number of *G. tachinoides*.

When the experiment 2 was repeated in Bapla near breeding sites, the result did not reveal statistically significant differences between the numbers of *S. damnosum* s.l. caught with HLC and any of the targets. Target size influenced catch density ($p < 0.0018$) during experiment (Figure 4). The small target + Fnet (mean catch = 188, 95% CI: [0, 532]) collected significantly more *S. damnosum* s.l. per target area than the large target + Fnet (CI95%: [0, 33]; $p < 0.0014$) but not than the medium target + Fnet (mean catch = 35, 95% CI: [3, 67], $p < 0.0501$). Days and locations
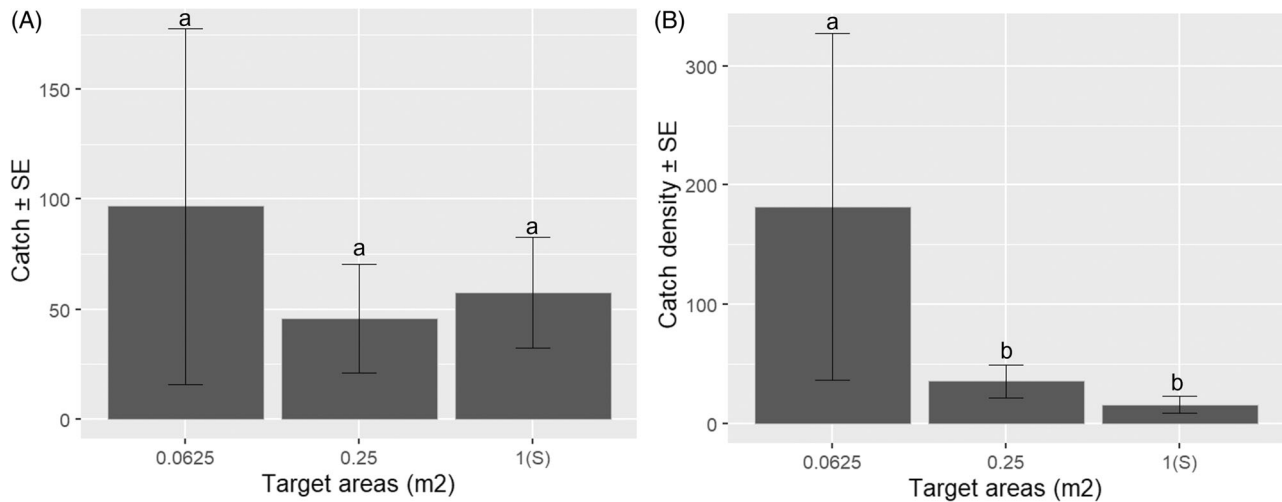
FIGURE 4 Comparison of decreasing size of Etarget and accompanied Fnet at Bapla in experiment 2. (a) Mean daily catch of *S. damnosum* caught by the decreasing size. (b) Comparison of the catch densities of the three targets. Different letters denote significant differences at $p < 0.01$ by negative binomial generalized linear models
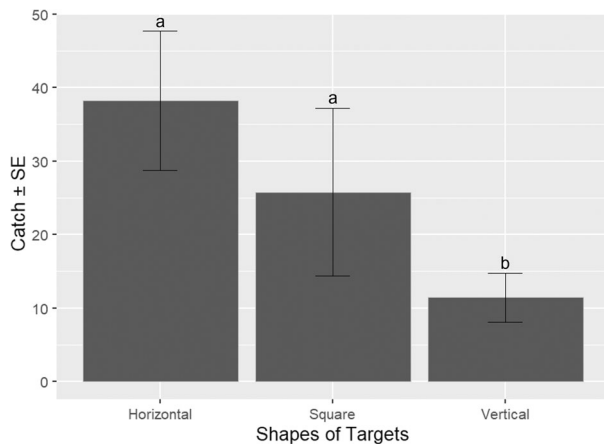

FIGURE 5 Mean daily catch number of *S. damnosum* caught by the three Etargets and accompanied Fnets (targets have approximatively same size area). Different letters denote significant differences at $p < 0.01$ by negative binomial generalized linear models

had no significant effect on catch or catch density. Target size significantly influenced the landing response ($F_{[2]} = 4.3$, $p < 0.02$) with the large Etarget having greater landing response, followed by the medium and the small Etarget.

The effect of target shape (experiments series B)

In this experiment 3, the HLC collected significantly more flies than all 4 traps combined. A comparison of trap catches showed that the square target collected 1.2× more *S. damnosum* s.l. than the horizontal target, and 2.6× more than the vertical target, but the differences were not significant. Similarly, no statistical difference between the number of *G. palpalis* collected by the targets or the number of *G. tachinoides* were observed.

In the second experiment of the series (Exp 4), where the Etargets were associated with Fnets, the horizontal rectangle Etarget + Fnet (mean catch = 38, 95% CI: [16, 60]) collected significantly more *S. damnosum* s.l. than the vertical rectangle (mean catch = 11, 95% CI: [3, 19] [$p < 0.02$]) but the difference with the square were not significant more than the square (mean catch = 26, 95% CI: [0, 52], n.s.). There was no significant difference between the square and the vertical rectangle (Figure 5). There was no significant difference in LR between shapes ($F[2] = 0.25$, ns). The Landing responses varied from 58 to 68%. For tsetse flies, the square target (mean catch = 30, 95% CI: [20, 40]) collected significantly more *G. palpalis*/day than the vertical (mean catch = 14, 95% CI: [7, 21], $p < 0.0448$) and the horizontal (mean catch = 15, 95% CI: [6, 24] $p < 0.0345$). No statistical difference between the number of *G. tachinoides* collected by the targets were observed.

Blackfly response to decreasing sizes of horizontal black targets (experiments series C)

Without Fnets (Exp 5), the large horizontal target (mean catch = 31, 95% CI: [0, 82]) collected significantly more *S. damnosum* s.l./day than the small target (mean catch = 3, 95% CI: [0, 6], $z = -3.4$, $p < 0.001$) but not the medium target (mean catch = 6, 95% CI: [1, 11], $z = -2.04$, $p = 0.1$). There was no significant difference between the medium and small target ($z = -1.14$, $p = 0.29$). When comparing the catch density, the small target collected the highest number of *S. damnosum* s.l./m², followed by the large horizontal target and then the medium horizontal target (Figure 6). However, these differences were not significant.

The three targets were compared with Fnets at Bodadiougou and Samandeni breeding sites (Exp 6). The results obtained in the two sites have been pooled for statistical analysis. The large horizontal target (mean catch = 7, 95% CI: [2, 15]) collected significantly more *S. damnosum* s.l./day than the small target (mean catch = 1, 95% CI: [0, 1] $p < 0.001$) but not than the medium target ($p = 0.22$) The small
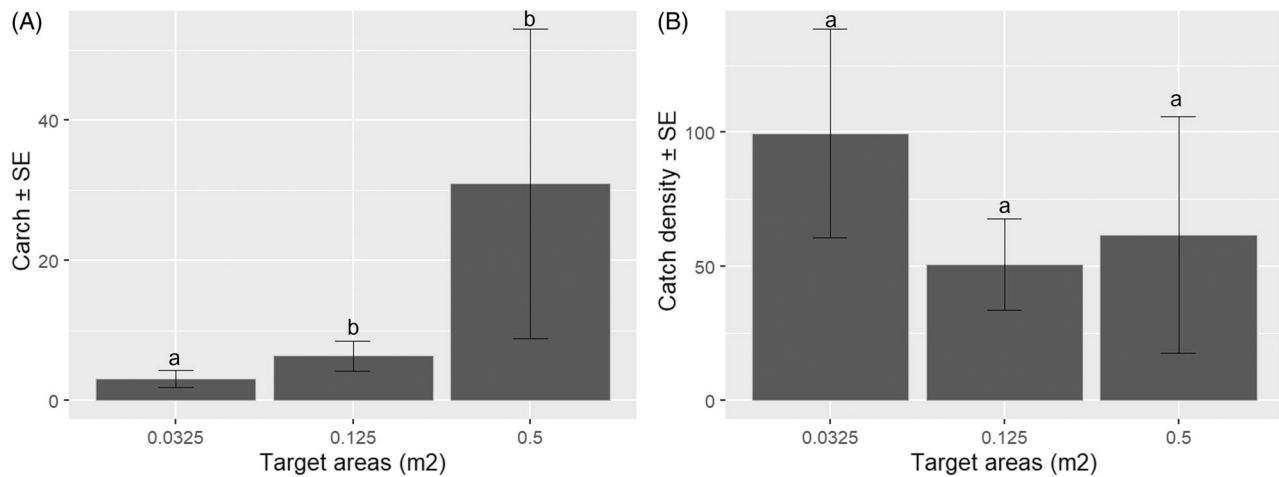
(A)

(B)

FIGURE 6 Comparison of decreasing size horizontal Etargets at Bodadiougou in experiment 4. (a) Mean daily catch of *S. damnosum* caught by the decreasing size. (b) Comparison of the catch densities of the three targets. Different letters denote significant differences at $p < 0.01$ by negative binomial generalized linear models
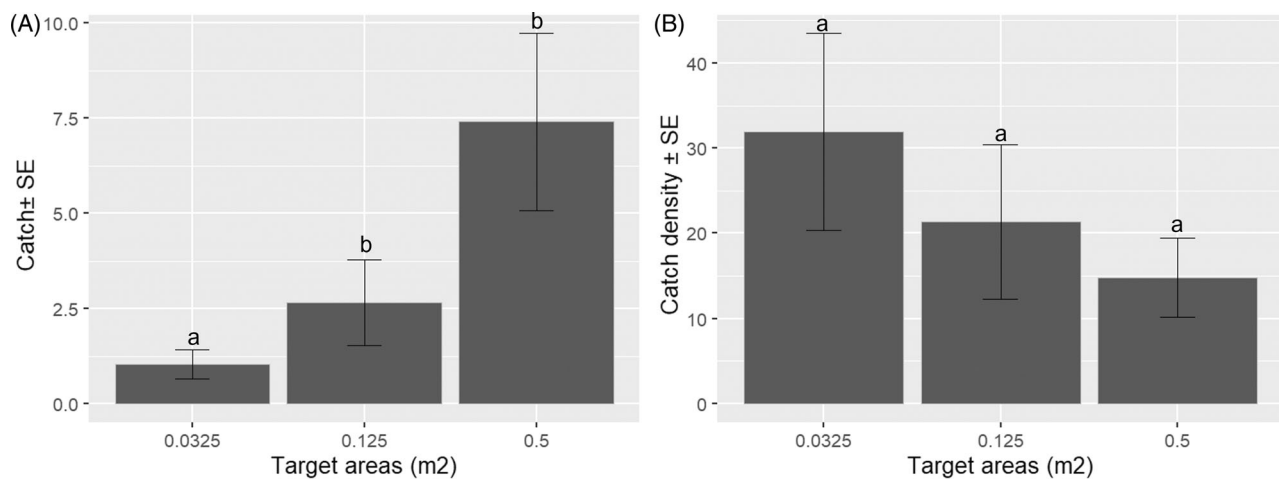
(A)

(B)

FIGURE 7 Comparison of decreasing size horizontal Etargets with accompanied Fnets at Bodadiougou in experiment 6. (a) Mean daily catch of *S. damnosum* caught by the decreasing size. (b) Comparison of the catch densities of the three targets. Different letters denote significant differences at $p < 0.01$ by negative binomial generalized linear model

target had the highest catch density followed by the medium and the large target, although these differences were not statistically significant (Figure 7b). Analyses of the landing response revealed that the size of target did not influence the percentage of *S. damnosum* s.l. landing on the Etargets ($F_{[2]} = 0.03$, ns). The Landing responses of the three targets were similar varying from 48% to 50%.

The effect of artificial odours on blackfly response (experiments series D)

Effect of carbon dioxide (Exp 7)

In this experiment, results showed that the targets baited with CO_2 collected the highest number of *S. damnosum* s.l./target/days compared to the unbaited targets (Figure 8). Thus, the target with a flanking net and baited with CO_2 caught 15 *S. damnosum* s.l./day/site (95%

CI: [2, 28]), significantly more than the 4 flies/day/site (95% CI: [1, 7]) collected with the unbaited target alone ($p < 0.04$), or the 3 flies/day/site (95% CI: [1, 5]) caught with the unbaited target accompanied by the Fnet ($z = 3.003$, $p < 0.01$); conversely, the catch difference between the CO_2-baited target, with (mean catch = 15, 95% CI: [2, 28]) and without Fnet (mean catch = 14, 95% CI: [3, 25]) were not significant ($z = 0.17$, $p = 0.99$). The target baited by CO_2 caught more flies than the target with accompanied Flanking net (mean catch = 3, 95% CI: [1, 5], $z = -2.829$, $p < 0.023$) but not than the unbaited one.

The target location also influenced the blackfly collections with position 1 catching the highest number of *S. damnosum* s.l. independently of target type. Its catch was significantly different than position 2 ($z = 2-3.017$, $p < 0.01$), but not position 3 or 4. Comparison of landing response of targets with accompanied Fnet revealed that CO_2 had no effect on the percentage of *S. damnosum* s.l. landing on the Etargets. The LRs for this experiment were 77.5% and 85.16% respectively for Etarget with Fnet and Etarget with Fnet baited by CO_2.

Targets collected consistent number of tsetse but there was no statistical difference between the number of *G. palpalis gambiense* collected by the targets; the number of collected *G. tachinoides* was too small for statistical comparisons.

Artificial odour bait experiments (Exp 8)

The results of this experiment revealed that targets baited with POCA blend caught consistently more *S. damnosum* s.l. than the other odours and unbaited target (Figure 9). Indeed, the POCA baited target (mean catch = 81, 95% CI: [0, 194]) collected 1.2× more *S. damnosum* s.l. than POCA and CO_2 (mean catch = 61, 95% CI: [0, 148]), 1.7× more than BG-lure and CO_2 baited target (mean catch = 46, 95% CI:
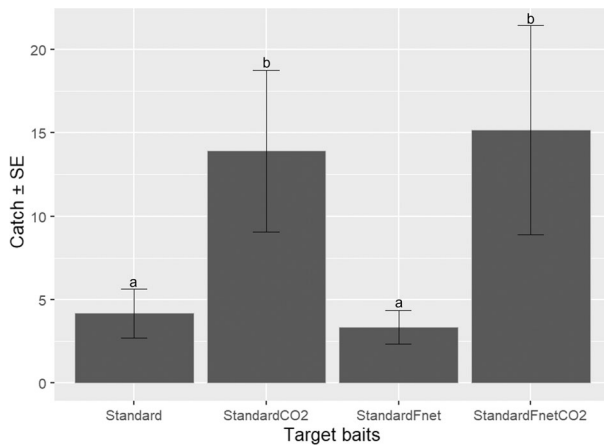

FIGURE 8 Assessment of the effect of the carbone dioxide and Fnet in experiment 7 at Bodadiougou. Mean daily catch of *S. damnosum* and se caught by the different treatments. Different letters denote significant differences at *p* < 0.01 by negative binomial generalized linear models

[0, 106]), 4.6× more than BG-lure single (mean catch: 18, 95% CI: [8, 26]), 2.4× more than CO_2 (mean catch = 34, 95% CI: [0, 78]) and 3.5× more than the control (mean catch = 23, 95% CI: [7, 39]). The target baited with BG-lure and CO_2 caught consistently more flies than the targets baited with BG-lure alone (which collected the lower number of flies of the six targets compared), the target baited with CO_2 only and unbaited target. However, statistical analyses revealed that there was no significant effect of odours on targets yields. In return, the locations of targets had a significant effect on the targets yields with location 1 collecting significantly more *S. damnosum* s.l. per days than each of other locations (*p* < 0.001).

DISCUSSION

The present study demonstrates the importance of better understanding the behaviour of *S. damnosum* s.l. towards different collection tools in xenomonitoring or vector control.

Implications for onchocerciasis xenomonitoring

In the assessment of fly response to varying target sizes, results showed that daily blackfly catches increased as the size of target increased. Except for experiment 2 at Bapla, the results of experiments 1 and 2 revealed that the large target collected more blackflies/day than the smaller targets. However, since there was no significant change in catch size as the size of target increased the smaller size could be also considered for blackfly collections. Also, the results of the landing responses show, independently of the study site, that the highest proportion of *S. damnosum* s.l. landed on the large targets, compared to the small ones. In experiments 3 and 4, the rectangular horizontal target of 0.5 m^2 area collected more blackflies per day than the rectangular or square shape.
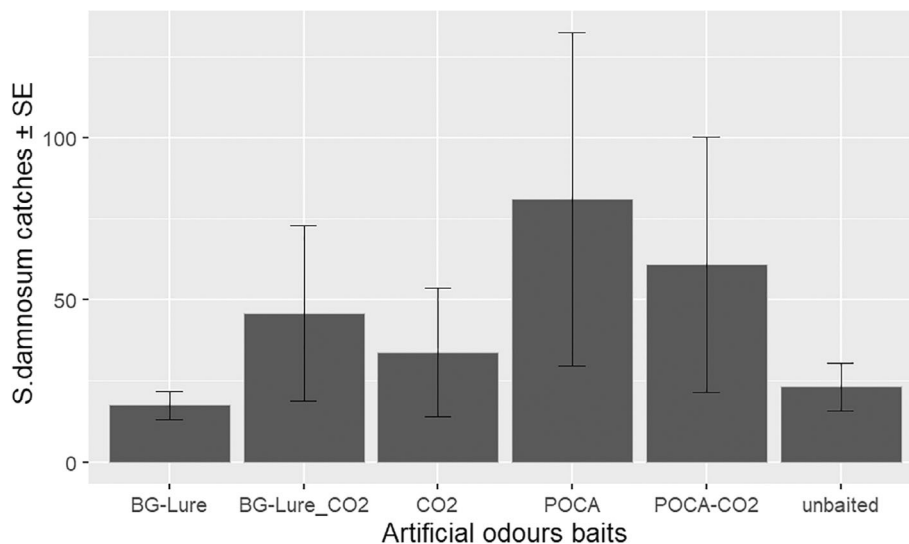

FIGURE 9 Assessment of *S. damnosum* response to different artificial odours in experiment 8 at Bapla. Mean daily catch of *S. damnosum* and se captured by the different targets

The comparison between several decreasing targets sizes of rectangular horizontal shape in experiments 5 and 6, showed that the large target of 0.5 m^2 collected the maximum number of *S. damnosum* s.l./day. Based on these results, we selected the 0.5 m^2 area horizontal target as the prototype target for the rest of the experiments.

Results also demonstrate that baiting such a trap with CO_2 would significantly increase yields as shown previously for blackflies and other vectors (Smallegange et al., 2010; Toé et al., 2014). In contrast, Experiment 6 results showed that adding flanking nets would have little effect on the target daily catches. In fact, apart from the small square target in Experiment 2, the results showed that the landing responses were generally greater than or equal to 50% with a peak of 77%–85% for the large horizontal shaped Etargets in Experiment 7. These results revealed that the majority of *S. damnosum* s.l. attracted by the Etargets end up landing on them. In other words, the cues that induce attraction in *S. damnosum* s.l are also likely to be involved in the landing on the host (or the target in our case). This landing behaviour is different from that of tsetse, for which the host cues involved in attraction are different from those involved in the fly landing (Vale, 2015).

In contrary to HLC (which collected only all hostseeking flies), the targets collected different physiological groups of *S. damnosum* s.l. However, hostseeking females were still the majority of target collections. This suggests that the target prototype described above could be a potential candidate for collecting *S. damnosum* s.l. flies in xenomonitoring framework. In the experiment with olfactory attractants, targets baited with POCA collected consistently more simuliid than those baited with BG-lure and/or simple CO_2. This is paradoxical, considering that POCA is an attractant extracted from cattle, (i.e., thus of animal origin) and likely to be less effective on *S. damnosum* s.l. (which is considered predominantly anthropophilic) than human odour. However, it is known that some species of the *S. damnosum* complex display a more zoophilic preference than their sibling species. Two species of Simulium are present in Burkina Faso namely *S. sirbanum* and *S. damnosum* s.s. and the populations of these two species cohabit in the breeding sites according to the seasons (Toé et al., 2014). In addition, several previous studies have shown that *S. sirbanum* has a more pronounced zoophilia than other savanna species (Fiasorgbor & Cheke, 1992; Sechan, 1984). Therefore, these results with olfactory attractants raise a crucial question: does the response of *S. damnosum* s.l. to different stimuli and/or olfactory attractants depend on its cytospecies? The experiment with olfactory attractants was conducted in September, that is, during the rainy season. Thompson (1977) reached the same hypothesis after comparing traps baited with goat and chicken odours in forest and savanna bioclimatic zones. The *Simulium damnosum* complex contains a range of species with diverse host preferences, that must be taken into consideration when seeking to identify host attractants.

Implications for vector control interventions against onchocerciasis

The smaller targets were the most efficient traps in terms of catch density. Therefore, in the context of vector control, with the aim to reduce Simulium populations with insecticide-impregnated targets, our results show that targets of 0.0625 to 0.25 m^2 are more efficient than targets of 1.0 m^2. This represents an important finding in terms of cost-effectiveness because with 8–16 times less material, targets remain just as efficient as large targets. In addition, experiments with shapes have shown that horizontal shapes are more attractive than other shapes commonly used in vector control such as the vertical rectangle or square. However, when comparing horizontal targets of decreasing sizes, the catch density of the small targets was much higher than those of the large target, but the difference was not significant implying that this size (0.0325 m^2) could be 'too small' to have an impact. Thus, based on our results, a horizontal target of size between 0.0625 and 0.5 m^2 could be a good prototype for a vector control tool against *S. damnosum* s.l. savanna cytospecies if impregnated with an appropriate insecticide to which the vectors are sensitive. Artificial odours such as CO_2 or POCA could be associated with this prototype to attract and kill the maximum number of blackflies.

Another important point of our results is the collection of certain physiological groups of *S. damnosum* s.l., such as males, new-emerged, and ovipositing flies, not currently considered in the vector control context. Indeed, these groups were collected mainly in the capture points located very close to rapids, and therefore breeding sites. It is indeed known that the new-emerged and males prefer the vegetation surrounding the rapid as resting places (Bellec & Hébrard, 1984; Crosskey, 1990). This could explain the success of certain vector control strategies such as slash-and-clear (Jacob et al., 2021). Given the importance of these physiological categories on the renewal of the population, an impregnated target exposed to these places could significantly reduce the populations of blackflies, and therefore the nuisance and or the transmission. However, these physiological groups are not of direct interest for parasite monitoring in vectors because the physiological stage of epidemiological importance is the hostseeking females. A target capturing these physiological groups would be of less use in xenomonitoring framework. Also, in the experiments with artificial baits, results revealed that catches were highly influenced by the location of the targets. Generally, positions with the highest *S. damnosum* s.l. catches were closer to the river (about 5–10 m from the river), brighter, and caught more new-emerged simuliids. In contrast, sites located over 10 m away from the river rapids (like positions within forest gallery bordering Comoé river in Bodadiougou sites) collected more host-seeking blackflies and tsetse. Therefore, ideal location would vary depending on whether the objective is: (i) to monitor or control blackflies, or (ii) the location is meant to target simultaneously Simulium and Glossina species, in an integrated vector control approach. In savanna area, locations targeting specifically Simulium spp should be placed near the river course (i.e., <10 m), whereas site locations targeting simultaneously both Simulium and *Glossina spp.* of Palpalis group (i.e., in an integrated vector control approach) should also be placed within the riverine forest, where these tsetse species are more abundant. Finally, in Bodadiougou breeding sites, targets collected as many tsetse flies as blackflies, implying that Tiny Targets (Tirados et al., 2015; Vander Kelen et al., 2020) commonly used

against tsetse flies could be used in an integrative approach in areas where these two vectors are co-endemic.

In summary, regarding the xenomonitoring framework where an effective trap should collect as many blackflies as possible, the results are disappointing: HLC consistently attracted and collected more blackflies than any of the traps tested. However, a comparison of different targets showed that black sticky targets with horizontal rectangular shapes ranging from 0.125 to 0.5 m^2 and baited with the POCA blend could be a viable alternative to HLC for surveillance of onchocerciasis. However, although they may provide proxies for entomological parameters on onchocerciasis transmission (e.g., biting rates, entomological inoculation rates, etc.) these attractants did not attract as many blackflies as human baits. In the vector control framework, our results showed that a horizontal target of size between 0.0625 and 0.5 m^2 could be a good prototype for a vector control tool against *S. damnosum* s.l. savanna cytospecies. And as, important number of tsetse flies were also collected during experiments, these small targets could also be considered for integrated control of onchocerciasis and trypanosomiasis vectors.

Study limitations

The major limitation in our study concerns the electrical grids used to evaluate the response of blackflies to the different target designs. Indeed, the electrical grids are experimental tools designed by Glynn Vale (2015) for the study of tsetse flies and therefore these tools were adapted to these vectors. Thus, the distance between the wires of a 'standard' electric grid is 4 mm, which is sufficient to capture tsetse flies with a wing length varying from 5 to 7 mm depending on the species. This spacing is not sufficient to cover all the *S. damnosum* s.l. whose wingspan varies from 3 to 6 mm depending on the species. Thus, during our experimentations we observed that some simuliids could pass between two electric wires of the grids and land on the target without being electrocuted. This observation was commonly made during the various experiments. We therefore argue that the collections of the electric grids were probably underestimated given this situation. In any case, sticky traps might be better suited for sampling onchocerciasis vector simulium. A second limitation in our study, is the difficulty to perform molecular identification of sibling species of *S. damnosum* s.s. and *S. sirbanum*. Although effective methods for differentiating the different species of the damnosum complex exist, there are still no reliable molecular biology methods capable of differentiating adults from the sibling species *S. damnosum* s.s. and *S. sirbanum*. Although this discrimination would make it possible to verify whether the cytospecies of a blackfly influenced its host-seeking behaviour or landing response. Another limitation is high catch dispersion of *S. damnosum* s.l. in some experiments. This variability could be attributed to the variable density of blackflies, environmental conditions, or the position of the traps, and constitutes a major challenge in the process of developing a collection tool. Finally, a visual cue that is just as important as size or shape is colour. Blue has been shown to be attractive to blackflies (Toé

et al., 2014). This target prototype is currently undergoing further evaluation in the field in Burkina Faso, in tests to evaluate the contribution of colour and odour to trap performance, and the final trap design will be reported in due course.

AUTHOR CONTRIBUTIONS

Lassane Koala, Roch K. Dabire, Philip J. McCall, Inaki Tirados, and Edward Thomsen conceived and designed the study. Lassane Koala and Achille S. Nikiema worked for the acquisition of data in the fields. Lassane Koala, Inaki Tirados, and Philip J. McCall analysed and interpreted of data. Lassane Koala wrote the first draft of the manuscript. Philip J. McCall, Inaki Tirados, Edward Thomsen, Roch K. Dabire, Achille S. Nikiema, and Lassane Koala revised critically the manuscript. All authors approved the submission of the final version.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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