

Evaluation of adhesive film as monitoring tool for riverine tsetse flies (Diptera, Glossinidae) in Burkina Faso

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Abstract

Tsetse flies (Diptera: Glossinidae) are the cyclical vectors of human (HAT) and animal (AAT) trypanosomiasis in sub-Saharan Africa, which constitutes a major constraint for human and animal health. Tsetse populations can be reduced using a variety of techniques. This studies aimed to validate the use of adhesive film as a measurement tool for riverine tsetse flies. For this study, two targets have been used. 1mx1m targets and small 0.25mx0.25m targets with adhesive film were compared using electrocuting sampling methods. Four treatments were compared for 12 days in a 4x4 latin square at Folonzo and Solenzo. Our results showed that the large targets (1mx1m black/blue/black BlkBBlk) (film or grid) captured significantly more *G. tachinoides* and *G. p. gambiensis* than the reduced targets (0.25mx0.25m BlkBBlk) ($p \leq 0, 001$) .For the large targets (1mx1m) *G. tachinoides* was significantly attracted on target covered adhesive film (27.91 flies/day) compared to the electric grid (19.65flies/day); $p \leq 0, 1$. The effect of adhesive film on color show also that the blue color of the 1mx1m BlkBBlk film was significantly more attractive than the target (1mx1m BlkBBlk) in the grid for *G. tachinoides*. No difference was observed between the targets with film and grid for the two colors (blue and black) for *G. p. gambiensis* in Folonzo and Solenzo. We conclude that adhesive film could be used as a strategic control tool against African Trypanosomiasis vectors.

Keywords: Tsetse fly control, adhesive film, cost, tsetse fly, Burkina Faso

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Introduction

Tsetse flies are the biological vectors of the parasites responsible for sleeping sickness in humans (HAT) and animal trypanosomoses (Nagana, TAA). These neglected tropical diseases are caused by infection with protozoan parasites of the genus *Trypanosoma* mainly transmitted by tsetse flies of the *Glossina* genus (1,2). For locating hosts, flies perceive visual and olfactory stimuli (3,4). In ecology, these observations on the behavior of tsetse allowed the development of control tools. Among these tools, there are the traps (biconic, monoconical) and targets in West Africa against riverine tsetse (*Glossina palpalis gambiensis*, *Glossina palpalis palpalis* and *Glossina tachinoïdes*) and (Ngruman, Epsilon) traps and targets against savannah species (*Glossina pallidipes*, *Glossina morsitans morsitans*) in Southern and Eastern Africa. The electric grid (5) is a capture device used for behavioral studies of tsetse fly species (6) and to test the effectiveness of various control tools (7). This device developed by Vale (1974b) was used to evaluate the effectiveness of traps and targets (8,9), and of olfactory attractants to improve the performance of lures (10,11) and to understand the responses of tsetse flies (7,12–14).

Nowadays, the transparent adhesive film (15,16) is used as a new capture device as a replacement for electric grid for behavioral and control studies (17–19). The transparent adhesive film was recently used to test, for example, the sticky panel against *G. austeni* (20), the Laveissière target (21) and traps (biconic and monoconica) against *G. palpalis* and *G. tachinoïdes* (15). It was also used as a sensitive monitoring device for the search for flies in low density situations (22).

This gradual replacement of the electrical grid by the adhesive film is due to logistical and economic constraints linked to its application in the field. Indeed, the operation of the grid requires the use of an accumulator (battery), a transformer and a tray for each model of screen to be tested. The duration of the experiments is often limited to 4 hours per day (active period of the tsetse fly) to save the battery and avoid complete discharge. In addition, a generator is required in the field to recharge the discharged batteries. At the same time, the constraints associated with moving and assembling the equipment, and its inefficiency during the rainy season, are a hindrance to its use for testing new lures.

Although transparent adhesive film has been adopted, few studies have been conducted on its effect (15) on the attractiveness of tsetse flies. Moreover, certain properties of the adhesive film such as shine could influence the visual response of flies and provide wrong results. Since the responses of *Palpalis* group, species are variable with respect to the size and shape (vertical vs. horizontal) of the targets (8,23). In this study we compare the effectiveness of the electric grid against the transparent adhesive film as capture tools for the study of riverine flies.

I. Material and Methods

1.1. Study sites

The experiments were conducted in two localities in Burkina Faso according to the level of conservation of the tsetse habitat and the presence of food hosts (domestic or wild) and tsetse species. At Solenzo (~12°14' N, 04°23'W), a location crossed by the Mouhoun River in the Banwa province in western Burkina Faso, *G. p. gambiensis* is present in highly anthropized habitats (10).

Agricultural activities are the main cause of the degradation of the gallery forest bordering the river. Pastoralism is practiced and mixed (Zebu*Taurin) breed cattle, also constitute the main source of blood meal for tsetse flies. A few wild animals such as reptiles (varanus, crocodiles and snakes), monkeys and hippopotamus found in the area, may represent a secondary source of blood meal.

At Folonzo (~09°54'N, 04° 36'W), the study was conducted in the protected reserve, and irrigated by the Comoé River. The gallery forest bordering the river is preserved and inhabited by two sympatric species, *G. tachinoides* and *G. palpalis gambiensis*. A diversity of floral and faunal species is encountered (19,24).

1.2. Capture methods

Electric grid (Figure 1) consists of two flat surfaces with electrical wires in copper spaced 8 mm apart and separated by a space. The space is sufficient to place the target to be tested. The grid is supplied with a potential difference (ddp) of approximately 40 kilovolts by a transformer and a battery (12 volts). The flies attracted by the screen to be tested are electrocuted and then fall into a tray filled with soapy water (to prevent the ants from carrying them away) before being collected.

According to the needs of the experimental studies, we carried out modifications of the grid. A reduction in the size of the grid was done to adapt it to scale models of target.

Electrocution devices (grids) designed for tsetse flies (12,25) and modified by Foil and Younger (2006) were used for cloth target evaluations in Louisiana. Grids consisted of a series of 0.18-mm diameter stainless steel wires placed 8-mm apart and strung vertically on the front and back sides of 1-m² aluminum frames (two electrified sides). The 1-m² targets to be evaluated could be inserted into the frames between the two electrified sides which intercepted and killed stable flies attracted by the targets (26). These are referred to as electric grid targets. Flies killed by electric grids are dropped to the ground into trays from which they can be removed and counted.



Figure 1: 1mx1m black/blue/ black target cloth inserted in electric grid

Adhesive film (Figure 2): transparent plastic material, Rentokil FE45/UK, available in rolls 30 cm wide, with one side being sticky (15,16). The target to be tested is stretched by stakes then the adhesive film is used to roll it up (on the two faces with a sticky film). The flies which are attracted to the fabric target are intercepted by the film and remain stuck when they land on it. Laboratory forceps are used to remove them from the film to identify and count species.



Figure 2: Target (1mx1m black/blue/ black) covered with adhesive film

1.3. Experimental design

To know the effect of the adhesive film, we inserted a square cloth target; 1 m², equal vertical rectangles of blue and black; standard target (21) in electric grid (1x1 m BlkBBlk with grid; Figure 1) and covered with adhesive film (1x1 m BlkBBlk covered adhesive film; Figure 2). To also study the effect of the adhesive film on the size of the target, the standard target was reduced by 1/16th (0.25x0.25 BlkBBlk or tiny target) (27). This tiny target was inserted in a modified electrical grid (0.25x0.25 BlkBBlk with grid) and covered with adhesive film (0.25x0.25 BlkBBlk with adhesive film)

Four treatments, 1x1 BlkBBlk inserted in electric grid, 1x1 BlkBBlk covered with adhesive film, 0.25x0.25 BlkBBlk in electric grid, and 0.25x0.25 BlkBBlk covered with adhesive film, were compared for 12 days in a 4x4 Latin square design of days' x sites x treatments, with 3 replicates. The experiments were carried out simultaneously from 8:00 am to 12:00 noon each day because *G. tachinoides* and *G. p. gambiensis* are more active at this time of day (28). To avoid the site effect, trapping positions were always ~100 m from one another. The experiments were conducted for the capture of *G. tachinoides* (Folonzo) and *G. p. gambiensis* (Folonzo and Solenzo). The captured flies were then counted by species and by sex.

1.4. Statistical analysis

The Genstat Discovery Edition 3 software (VSN International program) was used for the analysis of the data. The daily catches were transformed into log 10 logarithm data (catch + 1) to homogenize the variances, then the means of each treatment were subjected to an analysis of variance (ANOVA). When a significant difference was observed the Bonferroni pairwise test was applied to establish treatment differences. The catches were then detransformed into geometric means ($\exp(\text{catch} - 1)$). The performances of the capture tools were expressed as a percentage (capture index) and correspond to the ratio between the captures (geometric means) of the screen with adhesive film and those of the screen with electric grid (control). Significant differences were based on pairwise t-test using Bonferroni correction ($\alpha=0.05$).

II. Results

2.1. Density of Tsetse flies

A total of 1293 flies were caught in the two localities, with 81.05% (1048/1293) of the samples at Folonzo and 18.95% (245/1293) at Solenzo. Between the two sympatric species encountered at Folonzo, *G. tachinoides* was the most predominant (70.03%, 734/1048). However, only *G. p. gambiensis* (100%) was captured at Solenzo.

Comparison of electric grid vs. adhesive film

Effect of target size (1mx1m and 0.25mx0.25m)

The size effect showed that the large targets (1mx1m BlkBBlk) regardless of the tool (film or grid), captured significantly more than the tinnytargets (0.25mx0.25m BlkBBlk) for *G. tachinoides* ($p < 0.001$; Table 1) and *G. p. gambiensis* ($p < 0.001$; Tables 2 and 3).

Table 1: Detransformed mean catches and indices of increase relative to control (1mx1m B1kBB1k grid or 0.25mx0.25m B1kBB1k grid) of *G. tachinoïdes* (males and females) using targets with grid and adhesive film at Folonzo.

Targets	Gtm	index	Gtf	index	GtT	index
1mx1m B1kBB1k grid	8.91	1.00	9.86	1.00	19.65	1.00
1mx1m B1kBB1k film	11.02	1.24*	16.58	1.68	27.91	1.42*
0.25mx0.25m B1kBB1k grid	0.54	1.00	0.10	1.00	0.69	1.00
0.25mx0.25m B1kBB1k film	2.14	3.95	0.78	8.11	2.98	4.32
n	12		12		12	
p-value	<0.001		0.001		0.001	
sed	0.06		0.09		0.06	

Catches followed by * differ from the standard at 0.01 level.

Gtm: *Glossina tachinoïdes* male; Gtf: *Glossina tachinoïdes* female; GtT: *Glossina tachinoïdes*

Total catch. Sed: Standard error deviation. n : 12

Table 2: Detransformed mean catches and indices of increase relative to control (1mx1m BIKBBIk grid or 0.25mx0.25m BIKBBIk grid) of *G. p. gambiensis* (males and females) using targets with grid and adhesive film at Fofonzo

Targets	Gpgm	index	Gpgf	index	GpgT	index
1mx1m BIKBBIk grid	3.54	1.00	5.22	1.00	8.68	1.00
1mx1m BIKBBIk film	2.74	0.77	4.15	0.79	6.73	0.77
0.25mx0.25m BIKBBIk grid	0.28	1.00	0.64	1.00	0.90	1.00
0.25mx0.25m BIKBBIk film	0.85	3.01	0.35	0.54	1.09	1.22
n	12		12		12	
p	<0.001		<0.001		<0.001	
sed	0.1054		0.0997		0.1131	

Gpgm: *Glossina palpalis gambiensis* male; Gpgf: *Glossina palpalis gambiensis* female;

GpgT: *Glossina palpalis gambiensis* Total catch (male+female). Sed: Standard error deviation

Table 3: Detransformed mean catches and indices of increase relative to control (1mx1m BIKBBIk grid or 0.25mx0.25m BIKBBIk grid) of *Glossina palpalis gambiensis* (males and females) using targets with grid and adhesive film in Solenzo

Targets	Gpgm	index	Gpgf	index	GpgT	index
1mx1m BIKBBIk grid	2.88	1.00	3.54	1.00	6.28	1.00
1mx1m BIKBBIk film	3.44	1.19	3.91	1.10	7.99	1.27
0.25mx0.25m BIKBBIk grid	0.19	1.00	0.12	1.00	0.33	1.00
0.25mx0.25m BIKBBIk film	0.33	1.74	0.44	3.67	0.78	2.36
n	12		12		12	
p	<0.001		<0.001		<0.001	
sed	0.0891		0.117		0.1057	

2.2. Surface of catch

For 1x1 BlkBBlk target, *G. tachinoides* was significantly captured on target covered adhesive film (27.91 flies/day) compared to target inserted in the electric grid (19.65 flies/day) (likelihood-ratio test: $\chi^2 = 4.17$, $df = 1$, $p = 0.01$). Male *G. tachinoides* were significantly more attracted to the adhesive film screen (catch index 1.24) than to the electric grid (control): ($p < 0.05$). In contrast, captured females showed no difference between the grid and the adhesive film (catch index 1.68; $p > 0.05$).

Also, no difference was observed between these two capture devices for males and females of *G. p. gambiensis* at Folonzo (Figure 3) and Solenzo.

For the reduced size (0.25x025BlkBBlk), the target with adhesive film did not capture significantly more flies compared to the target with electric grid for the two sympatric species *G. tachinoides* and *G. p. gambiensis* (Figure 3).

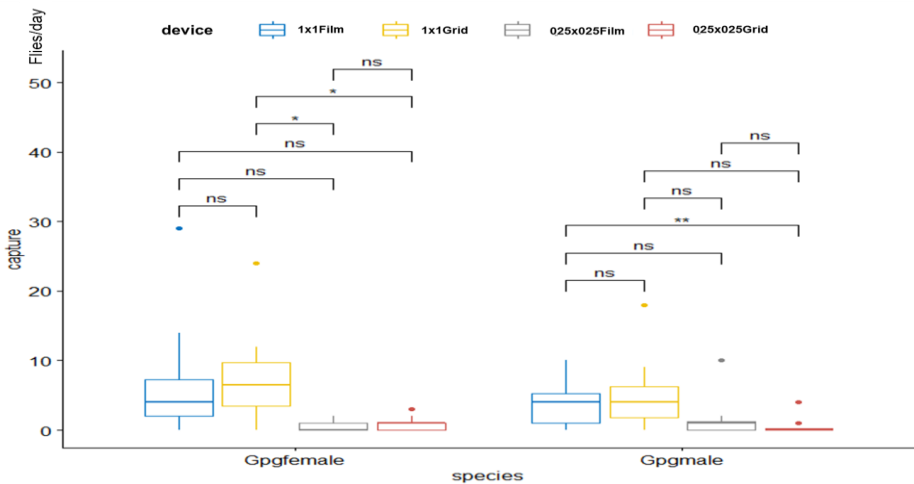


Figure 3: Captures of males and females of *G. p. gambiensis* at Folonzo. ns; indicates non-significance of the comparison between the different targets (captures device and size)

2.3. Effect of adhesive film on color

To understand the effect of the adhesive film on the blue and black color of the targets and the response of the flies, the catches of target: 1mx1m BlkBBlk Grid and 1mx1m BlkBBlk Film were compared. The results

showed that the blue color of the 1mx1m BlkBBlk film was significantly more attractive than that of the target (1mx1m BlkBBlk) in the grid for *G. tachinoides* in Folonzo ($p < 0.00297$; Figure 4). However, the comparison between the grid target and the film target for black color, has not shown a difference ($p = 0.1$) for the same species.

For the two colors (blue and black), no difference was observed between the targets with film and grid for *G. p. gambiensis* at Folonzo and Solenzo.

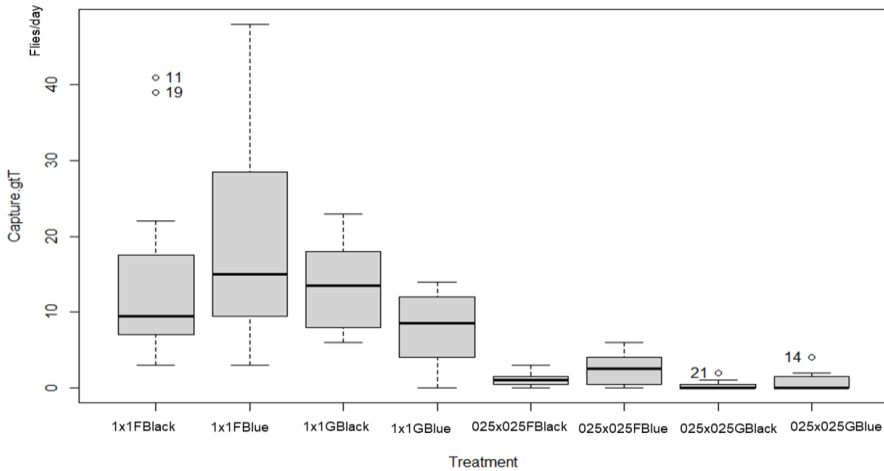


Figure 4: Median capture on blue and black sections of the target (by capture devices and size) for *G. tachinoides*

III. Discussion

Tsetse densities

This study was performed to evaluate adhesive film as a measurement tool for riverine tsetse flies. According Salou et al. 2012 and Rayaisse et al. 2015, Folonzo is the protected reserve, which may explain the tsetse density conservation in this locality. Another factor which could influence this result is the presence of several wild hosts species in this protected area, which constitute the main hosts of the tsetse (29).

Comparison of electric grid vs. adhesive film

Effect of target size

For the two tsetse species, the large targets captured significantly more than the reduced target.

Previous study carried out in eastern and southern Africa on species of the *morsitans* group found no correlation between catch and targets size (12). The reason for these differences in response between species is not known. These observations may be related to the habitat or food hosts of the flies. Savanna tsetse of the *morsitans* group feed primarily on wild and domestic mammals, which are generally larger than the reptiles that are the primary diet of the tsetse. *G. p. gambiensis* and *G. tachinoides* are considered opportunistic in their feeding habits, feeding on mammals, including humans as well as reptiles. The comparison between the two tools devices showed that the adhesive film can increase the captures of *G. tachinoides* species but does not improve those of *G. p. gambiensis* compared to the electric grid. Opposite results were found in other studies. Indeed, the adhesive film had halved the captures of *G. tachinoides* and *G. p. gambiensis* in Burkina Faso (15) and of *G. p. palpalis* in Côte d'Ivoire (9). This variability could be explained by extrinsic factors specific to lures (size, shape, color, contrast, location, tissue qualities, etc.) (12,23,30). Also, tsetse flies of the *palpalis* group seem to be more sensitive to visual aspects (31).

Surface of catch

The impact of the adhesive film on the behavior of the flies differed between the blue and black sections of the target. Capture of *G. tachinoides* with the 1mx1m target, was significant with the application of adhesive film in contrast to the electric grid. No difference in capture device was observed in males than females in both sites (Folonzo and Solenzo). We can then say that the addition of the adhesive film has no effect on sex

Effect of adhesive film on color

A difference in fly behavior was observed for the blue and black portions of the target. During the study, the blue color with the 1mx1m BlkBBBlk film was significantly more attractive than the target (1mx1m BlkBBBlk) of the grid for *G. tachinoides* in Folonzo and the comparison between the grid target and the film target for the black color, showed no difference for the same species. The opposite effect was observed for *G. tachinoides* where the adhesive film had no effect on the numbers landing on the blue section. In contrast, on the black section, the addition of the adhesive film reduced *G. tachinoides* captures by approximately two-thirds (9). Area-normalized fly captures were three times higher on the smaller device. This confirms the three times higher attraction per unit area recorded for *G. p. palpalis* on 0.25 m² black

cloth targets compared to 1 m² targets of the same color by Tirados et al., (2011) in Côte d'Ivoire. In contrast for both colors (blue and black), no difference was observed between targets with film and grid for *G. p. gambiensis* at Folonzo and Solenzo. These results are opposed to those observed by Kaba et al., (2014) where adhesive film significantly increased the number of flies landing on the blue section and adhesive film reduced captures by about three quarters on the black section. Other opposite field results using clear adhesive film squares as capture devices conducted in Ethiopia on *G. tachinoides* showed that the film itself when attached vertically is not attractive (32). Also, the field trial conducted in Angola in 2012 (Rayaissé com. pers) showed that landing of *G. p. palpalis* with a notable preference for landing on the black part depending on the type of trap (pyramidal).

In the state of available methods for capturing adults, techniques using traps and targets seem to be the most suitable.

Our study shows that large targets significantly caught more flies than small targets in Burkina Faso suggesting that they are more efficient sampling tools for *G. tachinoides*. The comparison of the targets with film and grid do not vary significantly in the two sites. Adhesive film could therefore enable us to improve the use of screens in terms of cost efficiency.

Conflict of interest

The authors have no conflicts of interest to report.

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