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## Survival and productivity of three strains of *Glossina palpalis gambiensis* for the selection of the best ones for mass rearing for better implementation of sterile insect technique

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### Abstract

A successful application of the sterile insect technique requires the release of sterile males of high biological quality. *Glossina palpalis gambiensis* strain, mass-reared at Centre International de Recherche-Développement sur l'Élevage en zone Sub-humide, Burkina Faso was used since 1980. This study aims to compare the biological parameters of this old strain with a newly domesticated strain and a hybrid of both strains to select the most productive strain. Three strains of *G. p. gambiensis* are reared at Insectarium de Bobo-Dioulasso : CIRDES, IBD and SEIB strains. Five batches were performed including three pure batches (Cirdes-Cirdes, Seib-Seib, and Ibd-Ibd) and two hybrid batches (Ibd-Seib and Ibd-Cirdes). The female survival varied by batch and sex ( $p < 0.001$ ) with the best survival rate obtained with the Seib-Seib batch and the lowest with the Cirdes-Cirdes batch. The strain IBD and hybrid batches (Ibd-Seib and Ibd-Cirdes) show better biological performance than the strain CIRDES.

**Keywords:** *Glossina palpalis gambiensis*, mass production, survival, fecundity, sterile insect technique

### Introduction

Tsetse flies are the only cyclic vector of trypanosomes, responsible for sleeping sickness or African human trypanosomiasis in humans and nagana or African animal trypanosomiasis in cattle in sub-Saharan Africa [1]. Tsetse infests 38 countries in Sub-Saharan Africa, hindering the development of sustainable and productive agricultural systems over more than 10 million km<sup>2</sup> [2]. Tsetse flies as a vector for trypanosomes in Africa leads to potential losses in agropastoral activities estimated at more than USD 4750 annually [3].

In view of these negative impacts on the development of agricultural production, several strategies and control campaigns were carried out against these diseases and their vectors. Attempts to create a vaccine against these diseases have failed because of the high antigenic variability of the parasite [4-6]; and existing trypanocides for chemotherapy are challenged by the development of trypanosome resistance [7]. As a matter of fact, vector control within the framework of Area-Wide Integrated Pest Management (AW-IPM) essentially based on the combination of chemical methods (chemotherapy, insecticide-impregnated screens and traps, ground and aerial spraying, epicutaneous treatment of animals) and a biological method, the Sterile Insect Technique (SIT), remains the most effective strategy [1]. Currently, there is an ambitious continental program aiming at the creation of sustainable tsetse and trypanosomiasis free zones via the African Union-Pan African Tsetse and Trypanosomiasis Eradication Campaign (AU-PATTEC), which implementation has started in six pilot countries including Ethiopia, Kenya, Uganda in East Africa and Mali, Ghana and Burkina Faso in West Africa [8]. Under the PATTEC, Burkina Faso has succeeded in reducing tsetse density in the target area by 95-99% [9]. In addition, a large mass rearing facility for tsetse, called the Insectarium de Bobo-Dioulasso (IBD), has been built and equipped to satisfy the various control campaigns in the West African sub-region with sterile males.

Tsetse rearing started in June 2016 at the IBD with *Glossina palpalis gambiensis* (Gpg) using flies from the Centre International de Recherche-Developpement sur l'Eleveage en zone Sub-humide (CIRDES) and the International Atomic Energy Agency (IAEA) insectariums. The Gpg strain was domesticated at the CIRDES insectarium under artificial conditions for about half a century (hereafter mentioned as Gpg-CIRDES) <sup>[10]</sup>. It was used effectively during the control campaign at Sideradougou, Burkina Faso <sup>[11]</sup> but appears to be losing competitiveness 30 years later <sup>[12]</sup>. Since the African Heads of State and Government decided in 2000 to embark a project with the ultimate goal to eradicate tsetse flies from the continent <sup>[8]</sup>, the decline in Gpg-CIRDES strain competitiveness could hinder the implementation of this objective.

In order to reduce a possible decline in performance of males of the old strain during release operations, IBD has domesticated a new strain of *G. p. gambiensis* (Gpg-IBD) from wild flies in 2016. IBD has also established a hybrid strain, resulting from the crossing of the old strain (Gpg-CIRDES) with the new one (Gpg-IBD) with the aim of producing a new hybrid strain that would improve the productivity traits of the Gpg-CIRDES strain by introducing the hardiness traits from the wild strain (Gpg-IBD). The aim of this study is therefore to evaluate and compare the production performance of these three strains of *G. p. gambiensis* in order to determine which strains could have better insectarium productivity and efficient competitiveness in the field.

## Materials and Methods

### Insectarium

The study was carried out at the Insectarium de Bobo-Dioulasso (IBD) localized at Darsalamy (11°03'32.4"N and 4°21'10.9"W), 15 km from Bobo-Dioulasso, Burkina Faso. Environmental conditions were maintained in the rearing rooms at 25 ± 1 °C, 75 ± 5% RH using air conditioners and humidifiers and a photoperiod of 12:12 light:dark <sup>[13]</sup> during the tests and for pupal incubation, feeding and fly monitoring.

### Tsetse strains

Three strains of *G. p. gambiensis* were used for the experiments: Gpg-CIRDES, IBD and CIRDES-Ibd. They are all in rearing at the Insectarium of Bobo-Dioulasso. The CIRDES strain originating from the CIRDES insectarium in Bobo-Dioulasso, the SEIB strain originating from the IAEA insectarium at Seibersdorf, Austria and the IBD strain from the insectarium of Bobo-Dioulasso.

The CIRDES strain was established in 1972 at Maisons-Alfort (France) using material collected in the field at Guinguette, near Bobo-Dioulasso, Burkina Faso <sup>[10]</sup>. The strain was transferred in 1975 to the Centre de Recherches sur les Trypanosomiasés Animales (CRTA), (CRTA and was later renamed CIRDES) <sup>[14]</sup>. In 2016, 53,972 adult male and female flies from the CIRDES colony were transferred to IBD where a colony was established for mass rearing for the needs of control programs in West Africa.

The SEIB strain was established at the Insect Pest Control Laboratory (IPCL) of the Joint FAO/IAEA Division, Seibersdorf, Austria in 2009 using 8,000 pupae from the CIRDES colony. The SEIB strain is mass reared to support the eradication program in Niayes in Senegal <sup>[15]</sup>. In 2017, 64,213 pupae from the SEIB colony were transferred to IBD where a colony was established for mass production.

The flies from CIRDES and SEIB strains originally possessed the same genetic material, the only difference remained the origin of the insectarium.

The IBD strain was domesticated at the IBD insectarium between August and December 2017 from wild flies collected in the field at Bama (11°23'48"N and 4°25'37"W), 30 km from Bobo-Dioulasso.

The flies used for the experiments were flies of approximately 186th generation for the CIRDES and SEIB strains and 3rd generation for the IBD strain.

### Experimental design

The experiments were conducted from August 2018 to January 2019. Five batches were constituted according to the type of crossing composed of three pure crosses (crossing between males and females of the same strain) and two hybrid crosses (crossing between females of the CIRDES and SEIB strains and males of the IBD strain):

Batch 1: the cross was carried out between males and females of the CIRDES strain and was named Cirde-Cirde.

Batch 2: the cross was carried out between males and females of the SEIB strain and was named Seib-Seib.

Batch 3: the cross was made between males and females of the IBD strain and was named Ibd-Ibd.

Batch 4: the cross was made between females of the CIRDES strain and males of the IBD strain and was named Cirde-Ibd.

Batch 5: the cross was made between females of the SEIB strain and males of the IBD strain and was named Seib-Ibd.

For each batch, 30 virgin females of 3 to 5 day-old were put into cage (13cm x 5cm x 8cm) covered with tulle of 2.5 mm of mesh and mated with 10 virgin males of 6-8 day-old. Each treatment was repeated three times.

The flies were fed daily with cattle blood collected from the slaughterhouse of Bobo-Dioulasso, with the consent from the slaughterhouse managers in order to obtain the blood samples from livestock. The blood was irradiated at 1 KGry in a Cobalt 60 irradiator (Model 812, S/N 002) and the bacteriological and biological qualities were tested before being used to feed flies.

Mortality was monitored every day (except Sunday) from the 1st day after the mating until the 47th day. Thus, cages containing flies were checked before feeding activities to remove dead flies, which were also separated by sex.

After mating, males and females were kept together in the same cage until the end of the monitoring. Cages were placed in individual cups and pupae were collected every morning. Pupa were separated into normal and aborted L3 and the normal pupae were weighed individually using an electronic balance (Hoaus Explorer precision 0.001 mg). Laying was accumulated per week for each cage and per batch in order to calculate pupae produce per female per 10 days (pf10d). The first larval period (time between female emergence and the production of the first pupae) was also recorded. The pupae were then incubated at 25 °C and 75% RH and the emerging flies were identified by sex and counted daily to assess the percentage of emergence.

### Data analysis

The statistical analyses and the figures were done with R Software (version 4.0.3) <sup>[16]</sup> using RStudio (RStudio, PBC, Boston, MA, USA, 2020) at a significance threshold set at 5%. The survival of flies was analyzed using Kaplan-Meier survival curves. The comparison between survival curves was done using the coxph model (in package Survival) <sup>[16]</sup> where

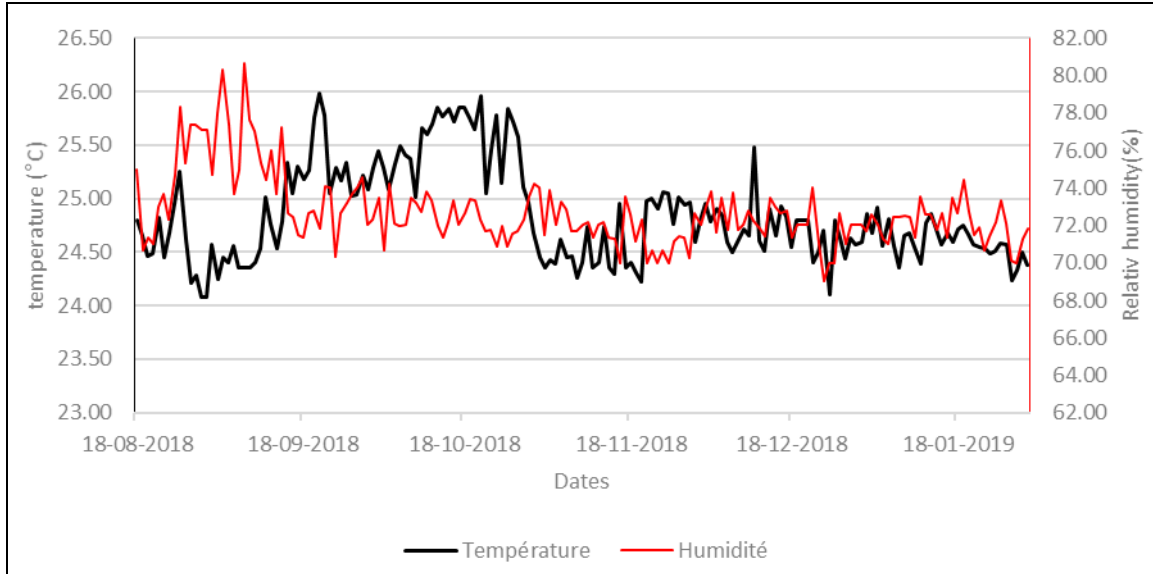
the batch and sex and their second order interactions were considered as explanatory variables and survival rate as the response variables.

For female fertility, the generalized linear mixed effect model was used to analyze the number of pupae produced per female every 10 days (pf10d) (with a Poisson distribution), the mean pupal weight (with a Gaussian distribution) and emergence rates with the lot and cage considered as a fixed variables [17].

**Results**

**Temperature and relative humidity during experiments**

Data recorded with Hobbo Data loggers® showed that the mean temperature ( $\pm$ sd) of the experimental room during experiments was  $24.84 \pm 0.45$  °C and the mean relative humidity ( $\pm$ sd) was  $72.72 \pm 0.45\%$  (Figure 1).



**Fig 1:** Temperatures and relative humidities recorded in the experimental room during the experiment

**Survival**

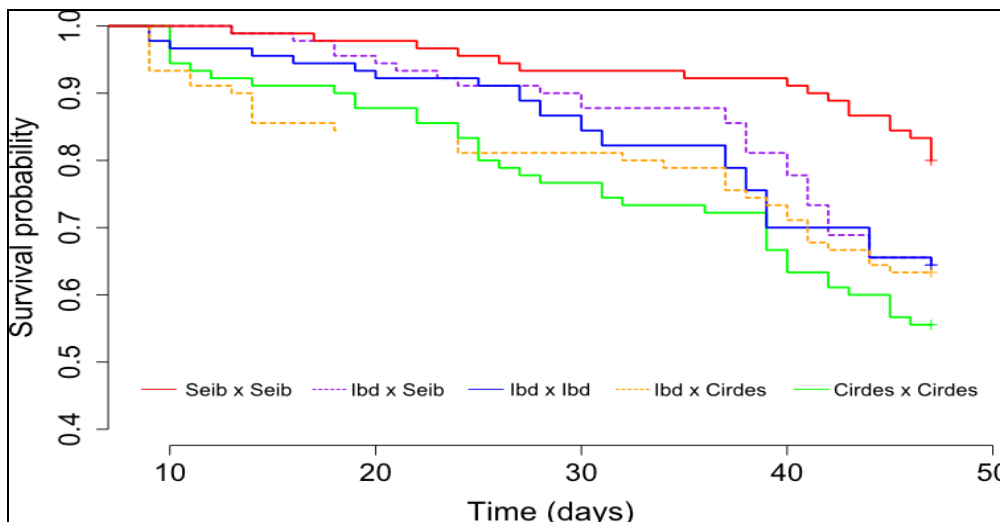
On 47 day post mating, the glm model analysis showed that the survival rate was significantly influenced by the batch ( $X^2_4 = 21.561$ ,  $p = 0.0002$ ) and sex ( $X^2_1 = 28.182$ ,  $dl = 1$ ,  $p < 0.001$ ). Females survived significantly longer than males, irrespective of the batch ( $p < 0.001$ ). For the females, survival of Seib-Seib was significantly higher than all the other batches ( $p < 0.02$ ), that were similar between them ( $p > 0.05$ ).

The hybrid batches Seib-Ibd and Cirdes-Ibd showed similar survival trends that the pur batches Ibd-Ibd and Cirdes-Cirdes, with the Cirdes-Cirdes batch from the old strain that recorded the lowest survival rate (Fig 2).

For the males, the survival of Seib males was similar to that of Ibd males ( $p = 0.1098$ ) but was statistically superior to Cirdes males ( $p = 0.03$ ).

**Table 1:** Summary of the cox model for the survival of female flies

Fixed effect	Coef	Exp(coef)	SE(coef)	Z-value	Pr(> z )
Cirdes-Cirdes	1.0211	2.7761	0.2840	3.595	0.000324***
Ibd-Ibd	0.7164	2.0470	0.2947	2.431	0.015075*
Ibd-Cirdes	0.6780	1.9699	0.2947	2.300	0.021425*
Ibd-Seib	0.7929	2.2097	0.2931	2.705	0.006824**



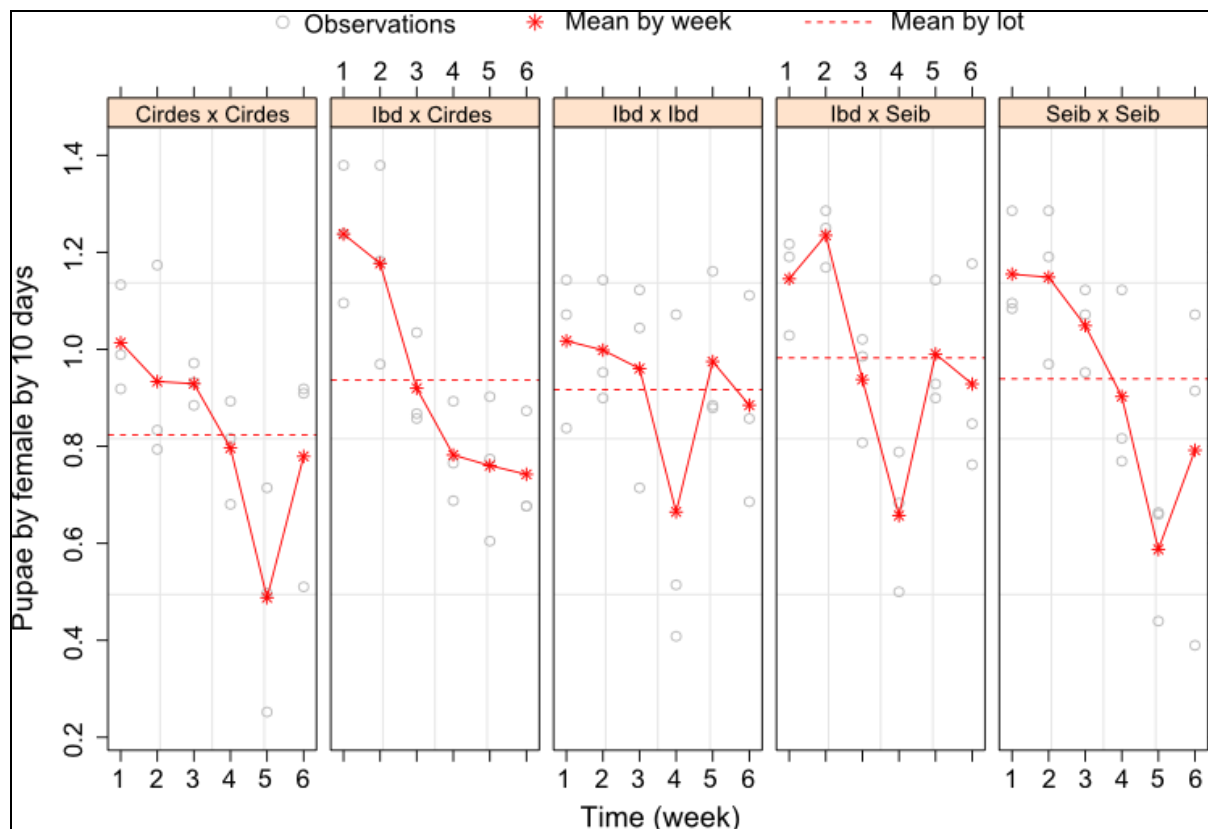
**Fig 2:** Survival curves of female flies in different batches

**Productivity**

The evolution of pupal production per female over each 10 days revealed a progressive trend in the different batches. The analysis shows that the lowest average productivity was observed in the Cirdes-Cirdes batch ( $0.82 \pm 0.23$  pupae per female over each 10 days) that was comparatively similar to those of the Ibd-Ibd ( $0.92 \pm 0.22$ ), Cirdes-Ibd ( $0.93 \pm 0.27$ ) and Seib-Seib ( $0.94 \pm 0.24$ ) batches ( $p > 0.2$ ), and significantly lower than that of the Seib-Ibd batch ( $0.98 \pm 0.22$ ) ( $p = 0.05$ ).

The average pupal mass obtained from the experimental females were significantly similar between batches ( $p > 0.05$ ; Table 2). The highest mass was observed in the Ibd-Ibd ( $22.74 \pm 2.00$  mg) and the lowest in the Cirdes-Ibd ( $21.20 \pm 2.62$  mg).

The pupal emergence rate was also similar between batches ( $p = 0.98$ ; Table 2). Indeed, the best emergence rate was observed in the Ibd-Ibd batch with ( $99.60 \pm 1.68\%$ ) and the lowest in the Cirdes-Cirdes batch ( $97.28 \pm 6.51\%$ ).



**Fig 3:** Pupal production per female at each 10-day interval.

**Table 2:** Summary of the best mixed effect model results for the pupae production and the emergence.

Trait	Fixed effect	Estimate	Std Error	z-value	Pr(> z )
Pupae per female per 10 days	(Intercept)	0.82326	0.05546	14.843	<2e-16 ***
	Cirdes-Ibd	0.11315	0.07844	1.443	0.1528
	Ibd-Ibd	0.09336	0.07844	1.190	0.2373
	Seib-Ibd	0.15901	0.07844	2.027	0.0458 *
	Seib-Seib	0.11574	0.07844	1.476	0.1438
Pupal mass	(Intercept)	21.3497	0.5191	41.125	<2e-16 ***
	Cirdes-Ibd	-0.1499	0.7342	-0.204	0.8387
	Ibd-Ibd	1.3968	0.7342	1.903	0.0605.
	Seib-Ibd	1.3752	0.7342	1.873	0.0645.
Adult emergence	(Intercept)	97.27734	1.04309	93.258	<2e-16 ***
	Cirdes-Ibd	0.91931	1.47516	0.623	0.535
	Ibd-Ibd	2.32584	1.47516	1.577	0.119
	Seib-Ibd	0.05311	1.47516	0.036	0.971
	Seib-Seib	0.98304	1.47516	0.666	0.507

Values with the same number of stars between rows and columns are not significantly different ( $p > 0.05$ ).

**Discussion**

One of the key factors that determine the success of an IPM program with a SIT component is the biological quality of the reared and release sterile males. The CIRDES strain, which was reared about over 50 years under artificial conditions in an insectarium and which showed its efficacy during the control campaign at Sidéradougu [11], appears to be declining

in competitiveness 30 years later [12]. This instigated our idea of domesticating of a new strain from wild flies (called IBD strain). This study evaluated the production performance of three strains of *G. palpalis gambiensis* and two hybrid strains resulting from the crossing of the pure strains in order to determine which strains could have good insectarium productivity and competitiveness in the field. The results will

enhance the development of an integrated pest management strategy with a SIT component that requires sterile males of very good biological quality<sup>[1, 18]</sup>.

Females of *G. p. gambiensis* showed a significantly longer lifespan than males regardless of the batch. This difference in survival between sexes is common to insects and appears to be related to genetic parameters<sup>[19]</sup>. Indeed, similar results had been obtained with the same tsetse species in previous studies under variable temperature conditions<sup>[20]</sup> and under different feeding regimes<sup>[21]</sup> and with mosquito species<sup>[22, 23]</sup>. Survival rates of female flies varied significantly between batches, where the Seib-Seib batch showed the best survival and the Cirde-Cirde batch had the lowest survival rate among all batches (pure and hybrids). The SEIB strain was initially derived from the CIRDES strain<sup>[15]</sup>. The difference in survival would come from the original insectarium (CIRDES and IAEA) because since their arrival at the IBD insectarium, a difference in production has been observed between the 2 strains which is better with SEIB strain (Insectarium of Bobo-Dioulasso, unpublished data). In addition to the environmental conditions that could vary between the two original insectarium, the condition and/or quality of the blood used for feeding the flies differs. Indeed, at the IAEA insectarium in Seibersdorf, frozen blood, after having undergone a 25-day feeding test to evaluate its effect on productivity and survival on a fly sample, is served to the SEIB colony<sup>[15]</sup>. Whereas at the CIRDES insectarium, fresh blood was used without this prior feeding test (CIRDES, personal communication).

Females of batch Ibd-Ibd showed intermediate survival time between the two pure batches Seib-Seib and Cirde-Cirde. Indeed, the CIRDES and SEIB strains were domesticated almost half a century ago<sup>[14]</sup> whereas the IBD strain was domesticated only one year ago. Despite this difference in establishment time in insectarium, this intermediate survival of the newly domesticated IBD strain reflects its good adaptation to rearing conditions. On the other hand, the CIRDES strain seems to experience a deterioration of biological quality over time.

The hybrid Cirde-Ibd and Seib-Ibd batches showed intermediate survival time between the pure batch Ibd-Ibd and Cirde-Cirde on the one hand and Ibd-Ibd and Seib-Seib on the other hand but without a significant difference. This seems logical as the females of Cirde-Ibd and Cirde-Cirde on the one hand, and Ibd-Seib and Seib-Seib on the other hand are from the same emergence groups respectively. In view of these results, hybrid strains (Cirde-Ibd and Seib-Ibd), resulting from the crossing between females of the old strains (CIRDES and SEIB) and males of the newly domesticated strain (IBD) could be established with a view to having strains that would have the productivity and adaptability to mass rearing conditions of the CIRDES and SEIB strains and the hardiness characters of the IBD strain. These new hybrid strains will be genetically composed of 50% of the genome of the old strains CIRDES and SEIB and 50% of the genome of IBD, then of 100% of the mitochondrial DNA of CIRDES and SEIB strains.

Our data indicated that the productivity of *G. palpalis gambiensis* females in hybrid batches was higher than that of pure batches but not significant. This result is encouraging and demonstrates a good compatibility between strains that were reared half a century ago with their congeners in the field. This means that hybridization between the old strains and the newly domesticated strain would improve their

productivity.

Also, the long confinement in artificial conditions seems to have no effect on the sexual behaviour of the flies but more on their production performance.

The good survival rate and the good productivity obtained with the SEIB strain seems to show that maintaining the colony over a long period of time in artificial rearing conditions has little impact on survival and productivity even if the CIRDES colony shows the opposite. Indeed, 5,000 pupae from the CIRDES colony were used to establish the SEIB colony and after 10 years of separation, their only difference remains the rearing insectarium. If at Insectarium de Bobo-Dioulasso, these 2 strains kept in the same temperature and relative humidity conditions with the same feeding regime continue to show a difference in production, this could not be due that the treatments in the original insectariums as explained above. This means that a colony could keep its production performances despite a long conservation in artificial conditions, if all rearing conditions remain optimal i.e. blood meal quality, environmental conditions. Nevertheless, the competitiveness of males should be investigated because data from previous studies conducted with the same CIRDES strain in its native environment in Burkina Faso showed a reduction in field competitiveness of sterile males 30 years later, i.e. a ratio of 10/1 sterile males to wild males in 1983<sup>[11]</sup> against 14.4/1 in 2012<sup>[12]</sup>. However, the same CIRDES strain has showed a very good performance in terms of competitiveness in the mangroves of Senegal (unusual habitat) in the context of the eradication campaign underway in the Niayes zone<sup>[24, 25]</sup>. In view of the above (behavior of the SEIB strain in our study and good competitiveness of the CIRDES strain in Senegal), the continuation of this study by evaluating the competitiveness of sterile males of the three strains (CIRDES, IBD and Cirde-Ibd) in semi-field cage and field in Burkina Faso is necessary to confirm or disprove the impact of maintaining the colony over a long period of time on the competitiveness of males.

Productivity of *G. p. gambiensis* females expressed as the number of pupae produced per female over 10 days ( $0.92 \pm 0.23$ ) was higher than that obtained in the Standard Operating Procedures for Mass-Rearing Tsetse flies with the same species, which was 0.6 pupae per initial female<sup>[23]</sup>. Similarly, this productivity is higher than that obtained with *Glossina pallidipes*, which was 0.57 pupa per female over 10 days<sup>[27]</sup>. There was no significant difference in production between batches. The better productivity obtained in this study compared to previous studies could be explained by the feeding regime to which tsetse fly were subjected, i.e. a blood meal every day during the experiment. Whereas in the study with *G. pallidipes*, the feeding regime was three times a week. Previous studies with *G. p. gambiensis*<sup>[21]</sup> and *G. morsitans morsitans*<sup>[28]</sup> on feeding frequency in relation to reproduction showed a positive correlation between feeding frequency and fertility. Indeed, the more the number of blood meals per week, the better the productivity is. For example with *G. p. gambiensis*, four times blood meals per week gave better results than three times per week<sup>[21]</sup>. Despite a relatively short domestication time of about 1 year, as well as survival, wild tsetse strain were able to adapt to such an extent that their productivity exceeded that of the CIRDES strain, even if it was not significant.

The results indicated a similarity between batches in mean pupal weight ( $22.11 \pm 2.17$  mg) and emergence rate ( $98.13 \pm$

4.41%). Overall, these results could attest that the good quality of pupae produced by the hybrids and good acclimatization of the flies of the new strain (IBD) is due to the artificial rearing conditions. However, another study will be necessary to characterize genetically and assess the symbiont populations of the three *G. p. gambiensis* strains (old, new and hybrid) in order to determine the causes of the deterioration of biological quality of the CIRDES strain with time.

### Conclusion

In view of the production and survival performance of the newly domesticated (IBD) and hybrid (Ibd-Seib and Ibd-Cirdes) strains and pending further studies to confirm the competitiveness of their male flies, mass production in insectarium could be initiated for future use in tsetse eradication programs in West Africa.

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