Agricultural Sciences, 2024, 15, 1391-1403
https://www.scirp.org/journal/as
ISSN Online: 2156-8561
ISSN Print: 2156-8553

Maximizing the Production of *Glossina palpalis gambiensis* Sterile Males in Mass Rearing by the Optimizing of the Sex Ratio

Ange Irénée Toé[1,2*], Soumaïla Pagabeleguem[2,3], Kiswend-sida Mikhaïlou Dera[2,4], Bénéwendé Aristide Kaboré[2], Zakaria Bengaly[5], Adrien Marie Gaston Belem[6]

[1]Centre Universitaire de Tenkodogo, Université Thomas Sankara, Ouagadougou, Burkina Faso
[2]Insectarium de Bobo-Dioulasso-Campagne d'Eradication de la mouche tsé-tsé et de la Trypanosomose (IBD-CETT), Bobo-Dioulasso, Burkina Faso
[3]Institut des Sciences de l'Environnement et du Développement Rural, Université Daniel-Ouezzin COULIBALY, Dédougou, Burkina Faso
[4]Österreichische JungArbeiterBewegung, Wien, Österreich
[5]Centre International de Récherche-Développement sur l'Elevage en Zone Subhumide (CIRDES), Bobo-Dioulasso, Burkina Faso
[6]Institut du Développement Rural, Université Nazi Boni (UNB), Bobo-Dioulasso, Burkina Faso
Email: *ange_20007@yahoo.fr

How to cite this paper: Toé, A.I., Pagabeleguem, S., Dera, K.M., Kaboré, B.A., Bengaly, Z. and Belem, A.M.G. (2024) Maximizing the Production of *Glossina palpalis gambiensis* Sterile Males in Mass Rearing by the Optimizing of the Sex Ratio. *Agricultural Sciences*, **15**, 1391-1403.
https://doi.org/10.4236/as.2024.1512076

Received: September 1, 2024
Accepted: December 3, 2024
Published: December 6, 2024

Abstract

Tsetse flies occur in much of sub-Saharan Africa as vectors of trypanosomes that cause human and animal African trypanosomosis. For its control, the Sterile Insect Technique based on mass rearing is currently used. But the improvement of tsetse productivity in mass rearing requires a better environment condition but also the control of productivity parameters. One of these important parameters is the choice of the ratio of females and males according to the targeted species. The aim of this study was to perform tests for getting the best female to male adults ratios in mass-rearing colonies of *Glossina palpalis gambiensis* for the purpose of optimizing the yield of sterile males. To achieve this, the mortality and fecundity for various male to female ratios (1:2, 1:3, 1:4, 1:8 and 1:10) on adult tsetse fly in routine rearing over 60 days after emergence was monitored and each experimental batch was replicated five times. Pupae production and fly mortalities were monitored daily except on Sunday. Females of the 1:4 ratio survived longer than those from 1:2 and 1:3 but similar to those from 1:8 and 1:10. The best survival was observed with flies from the ratio 1:10. The highest pupae per initial female per 10 days was observed with the ratio 1:4. The best *Glossina palpalis gambiensis* male to female sex ratio should be 1:4, due to the higher significant fecundity combined with lower mortality of females, in order to maximize the productivity of the

colonies and the yield of male flies that can then devoted to sterile insect technique projects.

Keywords

Glossina palpalis gambiensis, Mass Rearing, Sex Ratio, Survival, Productivity, Optimization, Sterile Insect Technique

1. Background

Tsetse flies are strictly hematophagous insects and are the biological vectors of African animal trypanosomoses (AATs) and African human trypanosomoses. (AHT) [1]. Today, 31 species and subspecies of tsetse fly have been identified [2] and this list could be extended in view of recent genetic studies [3] [4].

In West Africa, *Glossina palpalis gambiensis* is one of the important vectors of trypanosomes, flagellated hematozoan of genus *Trypanosoma* [5] [6].

G. p. gambiensis originates from the glossinian population of the Guinguette forest.

Guinguette classified forest and the Hippopotamus pond in Satiri in the Haut-Bassin region of Burkina Faso in the 1970s. Indeed pupae of the said species were collected and sent to the entomology laboratory of the *Institut d'Elevage et de Médecine Vétérinaire des Pays Tropicaux* (*IEMVT*) in Maison Alfort, France. From this laboratory, ten thousand twenty-four (10,024) pupae were sent to the CRTA (now CIRDES) for the start of tsetse fly rearing in March 1975 [7].

African animal trypanosomiasis (AAT) is a serious livestock disease that affects livestock production in sub-humid and humid regions of Africa [8].

As regards the negative impacts on agricultural production, several control strategies and campaigns were conducted against nagana or Animal African Trypanosomiasis (AAT) and their vectors, including vector control [9] [10]. Indeed, vector control within the framework of Area-Wide Integrated Pest Management, based mainly on a 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 [11]. SIT involves the mass rearing of sterilized adult males who are released into the wild to compete with wild males and mate with wild virgin females. This mating is not productive and leads to the reduction or elimination of the population [12].

An ambitious continental program called the African Union-Pan African Tsetse and Trypanosomosis Eradication Campaign (AU-PATTEC) aims to create sustainable tsetse and trypanosomosis-free zones. Thus, the first pilot phase of PATTEC was implemented in Ethiopia, Kenya, Uganda in East Africa and Mali, Ghana, and Burkina Faso in West Africa [13]. In Burkina Faso, the project performed an 83 to 92% reduction of *Glossina palpalis gambiensis* and *G. tachinoides*

densities on a 40,000 km² area through an integrated control campaign including insecticide targets, traps and cattle, sequential aerial treatment (SAT) and the mass treatment of livestock using trypanocides, from June 2006 to December 2013 [14]. Moreover, a mass-rearing facility, named "Insectarium *de Bobo-Dioulasso* (*IBD*)", was set up with capacities to produce about 1,000,000 sterile males weekly in order to provide sterile males to Burkina Faso programme, and to other countries PATTEC campaigns in west Africa in which a sterile insect technique (SIT) is performed [14]. In this facility, tsetse fly rearing started in June 2016 with *Glossina palpalis gambiensis* and in December 2022, the total colony population reached 650,000 females [15].

In addition, in 2005, the Government of Senegal initiated under the PATTEC initiative, a tsetse flies eradication program in the Niayes area using area-wide integrated pest management approaches with the SIT component [16] [17]. For the SIT component, since 2017, an agreement has been made with the IBD to mass-produce *G. palpali*s gambiensis in order to provide the Senegal program with 50,000 sterile male pupae weekly. Furthermore, the Burkina programme plans to implement SIT in defined and isolated areas along the Mouhoun River in its intervention zone performed.

As regards the colony size and the needs of sterile males for the active and near future programmes (Burkina, Ghana, Mali, Chad, etc.), the yield improvement for sterile males was absolutely requested. Indeed, the ratio of released sterile male flies to fertile wild male flies must be as high as possible to induce high sterility in wild females [18].

At IBD, the *G. palpalis gambiensis* colony is maintained with a mating sex ratio of 1:3 males to females. A similar recent study was carried out at the National Institute for the Control and Eradication of Tsetse and Trypanosomosis (NICETT), Ethiopia on *G. fuscipes* and *G. pallidipes* recommended to use 1:4 male to female sex ratio to the whole colonies (instead of 1:3) and this improved the yield of sterile males for the eradication project [19].

Therefore, the same framework was implemented to perform various ratios of male to female adults in mass-rearing colonies of *G. palpalis gambiensis* in order to provide the best yield of sterile males while ensuring better productivity of the colony.

2. Materials and Methods

2.1. Insectarium

The study was implemented at the "*Insectarium de Bobo-Dioulasso (IBD)*" located in the village of Darsalamy, 15 km from Bobo-Dioulasso, Burkina Faso (11˚03'32.4"N and 4˚21'10.9"W). The rearing rooms are equipped with specific materials such as air conditioners, humidification and cooling devices to maintain specific environmental conditions at 25 ± 1˚C, 75 ± 5% RH and a photoperiod of 12:12 light: dark during the tests and for pupal incubation, feeding and fly monitoring [20]. Data loggers are placed inside rooms and are programmed to display temperature and relative humidity every minute and to record data every 30 min.

2.2. Tsetse Species and Strain

The sex ratio experiments were performed with *G. palpalis gambiensis* flies from the laboratory colony maintained at the IBD and fed 4 times per week [15] with defibrinated and irradiated bovine blood using *in vitro* silicon membrane heated on a feeding plate at 36 ± 1˚C [21]. The colony was derived from an original strain from Maisons-Alfort (France) in 1972 using local pupae collected in the field at Guinguette, near Bobo-Dioulasso, Burkina Faso to make it easier for them to emerge and transferred to "*Centre de Recherche sur les Trypanosomiases Animales (CRTA)*" in Burkina Faso in 1975 [22] renamed later on Centre International de Recherche-Developpement sur l'Elevage en zone Subhumide (CIRDES) [7]. In 2016, 53,972 adult flies of this CIRDES colony were transferred to the IBD in order to set up a colony for mass production as regards the objective of the insectary [15].

2.3. Experimental Design

The study was run from November, 2020 to April, 2021 and included 2,025 *G. palpalis gambiensis* adults (1,600 females and 475 males). To achieve the objective of the study, five sex ratios male:female were implemented *i.e.* 1:2, 1:3, 1:4, 1:8 and 1:10. Three days-old virgin females were mated with 6 days-old virgin males (the time that the flies become sexually mature) according to sex ratio and put into a cage (13 × 5 × 8 cm) covered with tulle of 2.5 mm of mesh. For each sex ratio, 5 cages were used and each cage constituted a replicate. The number of males and females according to sex ratio is detailed in Table 1. Cages were maintained under the same conditions (environmental conditions, feeding and management). Males and females remained together until the end of the experiment.

Table 1. A number of females and males *Glossina palpalis gambienis* were tested according to the sex ratio.

Sex ratio (male:female)	Males	Females	Number of cages
1:2	30	60	5
1:3	30	90	5
1:4	10	40	5
1:8	10	80	5
1:10	5	50	5

2.4. Mortality

Mortality monitoring consisted of checking each cage for dead tsetse flies from the first day after mating until 60 days. Mortalities were recorded per sex ratio, cage and fly sex daily except on Saturdays and Sundays. Death flies were separated by blood mortality and starvation.

2.5. Fecundity

Mating cages were set up in individual larviposition cups and the pupae were collected every morning except on Saturdays and Sundays. Viable pupae were separated from aborted larvae and recorded per sex ratio and cage. The viable pupae were

weighed individually per sex ratio and cage using an electronic balance of 0.0001 mg sensitivity and automatic calibration (Sartorius MSE2 7S-000-DM Cubis Ultra [MD]). The pupae produced per female over 10 days (pf10d) was also calculated [23].

The pupae produced were put in emergence cages (30 × 25 × 15 cm) and set up in the incubation room at 25 ± 1˚C, and 75 ± 5% RH until adult emergence. Emerged flies were removed and identified by sex to calculate the percentage of emergence per sex ratio [23].

2.6. Data Analysis

The statistical analyses were performed using R Software (version 4.0.3) [24] with Rstudio. The Shapiro-Wilk test was used to test the normality of data and Tukey's test was applied. The survival of flies was analysed using the Kaplan-Meier survival curves and was compared using the coxme model [25] for the sex ratio, the sex and their second order interactions were considered as explanatory variables, the cage number was used as a random effect, and survival rate as the variable response. The significant interactions were analyzed using the emmeans function (in package *emmeans*) [26]. The number of pupae produced per female over 10 days, the pupal mass and the emergence rate were compared between sex ratios using the generalized linear model provided by the post hoc Tukey tests (glht function in package *multcomp*) [27].

3. Results

3.1. Flies Survival

The survival rate was significantly influenced by the sex ratio ($F = 188.839$; $df = 1, 26$; $p < 0.001$) and sex ($F = 45.94$; $df = 4$; $p < 0.001$). In addition, females survived significantly longer than males, irrespective of the sex ratio ($P < 0.001$). For the females, the comparison between sex ratios showed that the best survival was observed with flies from the ratio 1:10 (**Figure 1**), followed by the ratio 1:4 and a significant difference was found between the two sex ratios ($p = 1.12e\text{-}6$; **Table 2**). The other ratios 1:2, 1:3 and 1:8 showed a survival significantly lower than the previously cited ($p < 0.001$, **Figure 1**). However, there was no significant difference between the survival rates of the 1:2, 1:3, and 1:8 ratios (**Table 2**). For the males, survival was similar in terms of the sex ratio ($P > 0.2$; **Table 2**).

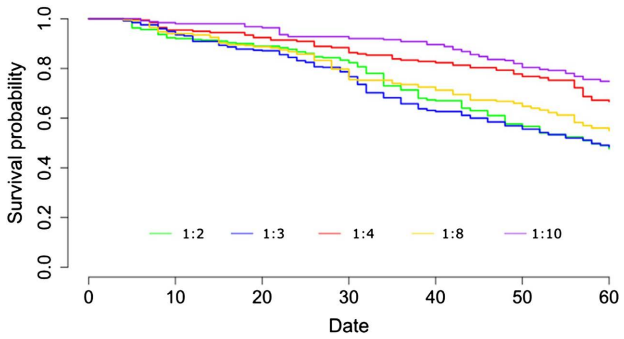

Figure 1. Survival of the female flies by sex ratio (male:female).

Table 2. Summary of the best cox model for the survival of flies per sex after 60 days. The sex ratio 1:4 is here considered as the reference level for females and males.

Traits	Fixed effect	Coef	Estimate	SE	T value	Pr (>|t|)
Female	ratio 1:8	−0.03382	0.96675	0.09540	−0.355	0.722958
	ratio 1:2	0.32212	1.38005	0.08603	3.744	0.00018***
	ratio 1:3	0.38557	1.47046	0.07917	4.870	1.12e-06***
	ratio 1:10	0.15981	1.17329	0.08091	1.975	0.048257*
Male	ratio 1:2	0.06650	1.06877	0.16579	0.401	0.688
	ratio 1:3	0.19579	1.21627	0.16542	1.184	0.237
	ratio 1:8	−0.02111	0.97911	0.20376	−0.104	0.917
	ratio 1:10	−0.15894	0.85305	0.24666	−0.644	0.519

Abbreviation: Coef, coefficient; SE, standard error. Significance: *** P ≤ 0.001; * P ≤ 0.05 (these apply to values above)

3.2. Productivity

The productivity was not significant according to the sex ratio (F = 2.912; df = 4; P = 0.02). The sex ratio with the best productivity was 1:4, which gave 0.71 pupae per female over 10 days (Figure 2). However, this production was not significantly different from those of the ratios 1:2 (0.62; p = 0.28) and 1:10 (0.70; p= 0.87). The significant lower fecundities (pf10d) were observed for the ratios 1:3 (0.51; p = 0.02) and 1:8 (0.48; p = 0.006; Table 3).

The highest pupal mass was observed for the ratio 1:10 (23.09 ± 0.89 mg; Figure 3). It was significantly higher (p < 0.03; Table 3) than the other ratios 1:2 (22.36 ± 2.15 mg), 1:3 (22.33 ± 2.08 mg) and 1:4 (22.46 ± 1.27 mg) and marginally higher than for 1:8 (22.59 ± 1.48 mg; p = 0.07). However, the emergence was significantly lower (p < 0.001) for the ratio 1:10 than that for the other ratios (Figure 4).

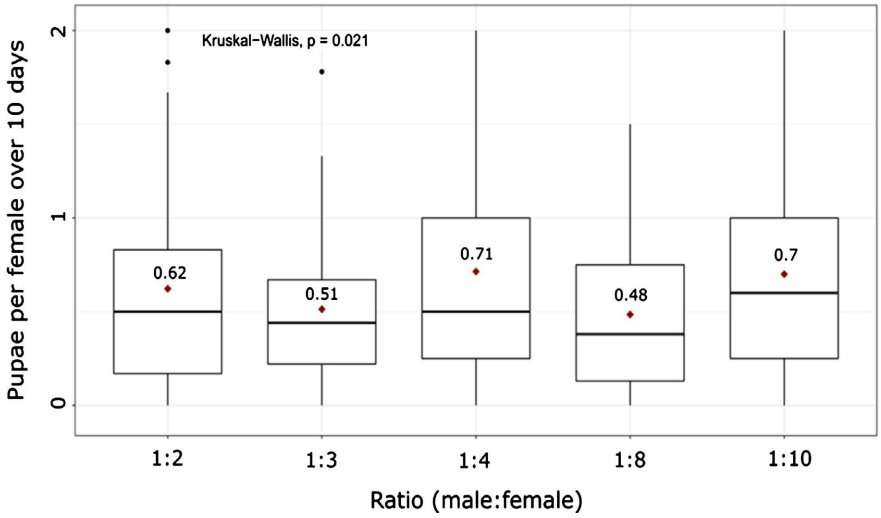

Figure 2. Number of pupae per female over 10 days. The red points give the mean value for each sex ratio.

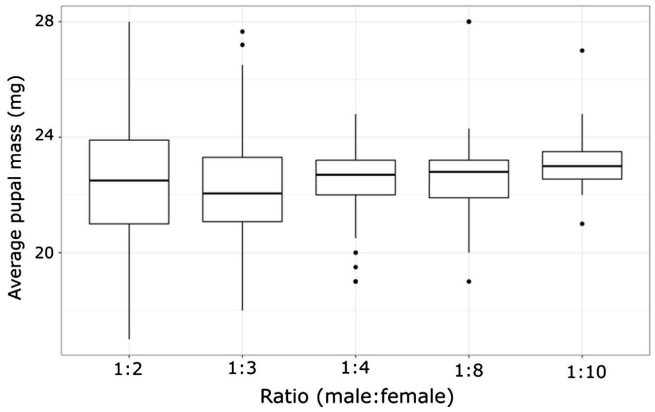

Figure 3. Average pupal mass according to the sex ratio.

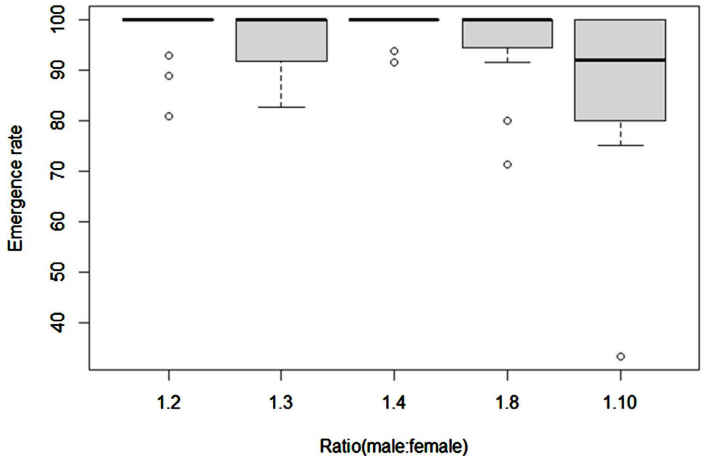

Figure 4. Emergence rate according to the sex ratio.

Table 3. Summary of the best mixed effect model results for pupae production and adult emergence.

Traits	Fixed effect	Estimate	Error	T value	Pr (>|t|)
pf10d	Intercept	0.71389	0.05989	11.921	<2e−16***
	ratio 1:2	−0.009232	0.08593	−1.704	0.28325
	ratio 1:3	−0.20016	0.08593	−2.329	0.02030*
	ratio 1:8	0.23194	0.08469	−2.739	0.00642**
	ratio 1:10	−0.01389	0.008469	−0.164	0.86981
Pupal mass	Intercept	23.09	0.1961	117.773	<2e−16***
	ratio 1:2	−0.7345	0.2932	−2.505	0.0128*
	ratio 1:3	−0.7642	0.2876	−2.657	0.0083**
	ratio 1:4	−0.6349	0.2876	−2.207	0.0280*
	ratio 1:10	−0.5009	0.2783	−1.800	0.0729
Adult emergence	Intercept	98.958	1.965	50.356	<2e−16***
	ratio 1:2	−1.506	2.815	−0.535	0.593888
	ratio 1:3	2.903	2.779	−1.045	0.298906
	ratio 1:8	−2.783	2.779	−1.405	0.298906
	ratio 1:10	−11.151	2.779	−4.012	0.000121***

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

4. Discussion

Tsetse flies are viviparous with too low production, *i.e.* at temperatures of about 25˚C, a female gives birth every 9 to 10 days a fully developed larva except the first which is laid 18 to 20 days after the fly is out of the puparium [28]. Thus, for mass-rearing facilities such as IBD with an objective to support different eradication campaigns in tsetse sterile males, it is important to maximize the production of the tsetse colonies and yield of sterile males to be able to satisfy the needs. This study has been carried out to assess some biological parameters of *G. palpalis gambiensis* adult flies mated in cages at various sex ratios in order to determine the best sex-ratio which is able to enhance the colony production and yield of sterile males.

4.1. Survival

One explanation for the fact females survived longer when they were mated in a ratio 1:4 comparatively to sex-ratios 1:2 and 1:3 (ratio used on the colony) could be the fact that in cages with low ratios of female flies per male, female flies were disturbed by mating attempts, reducing their longevity. These observations are in line with previous data found in cage conditions with *G. morsitans morsitans* [29], *G. fuscipes fuscipes* and *G. pallidipes* [19]. For tsetse flies, in general, one mating is enough for life time reproduction [28], although exceptions exist, for example with *G. fuscipes fuscipes*, two or three mating have been observed in the wild [30]. In confined areas such as rearing cages, male flies struggle to mate females repeatedly since with age the male mating competitiveness increases [31]. The consequence is that this disturbance results in stress, loss of energy, abortion or even death [29]. Altogether these factors reduce survival in cages of flies with 1:2 and 1:3 ratios. However, the results showed similar survival between female flies from ratio 1:4 and 1:10.

Another explanation of the survival difference between ratios could be the difference in the density of flies per experiment cage (13 × 5 × 8 cm). Indeed, the total number of female flies contained per cage was 45 and 50 for the ratios 1:4 and 1:10 while that was 90 and 120 for the ratios 1:2 and 1:3 respectively. A previous study was found with *G. palpalis gambiensis* using the ratio 1:3 that the high density of 120 flies per cage (90 females) appeared to cause high mortality (1.4 ± 0.3) comparatively to a density of 60 flies per cage (45 females) which daily mortality rate was (0.9 ± 0 3) [32]. The future ratio studies should take into account the density parameter to avoid its effect.

4.2. Productivity

Females from the sex ratio 1:4 showed the highest fecundity. The pupae per initial female over 10 days (0.7 ± 0.57) was slightly higher than that obtained in the Standard Operating Procedures for Mass-Rearing Tsetse flies with the same species, which was 0.6 pupae per female over 10 days [23]. This means that using these two sex ratios could improve the productivity than keeping the ratio 1:3.

However, the productivity remains lower than that obtained by [33] using the same *G. palpalis gambiensis* with the ratio 1:3 in the same insectary (0.82 ± 0.23) [33]. The difference between these two studies is the density of flies per cage. The study performed in 2021 used the same sex ratio 1:3 in a very low density (30 females × 10 males) versus (90 females × 30 males) for the present. Does that mean that it's the density of the flies that impacts the productivity more than the ratio? This is a question that still needs to be answered. The reduced fecundity at a higher ratio (1:8) might be due to an insufficient number of males to mate all females at the required time, since generally females refuse mating after fifteen to twenty days old [30]. Non-mated flies would then die without giving any pupae. The exceptional case of the ratio 1:10 could be explained by the lower fly density per cage.

The average weight (22.16 ± 1.88 mg) and emergence rate (95.26 ± 9.45%) found in these experiments could attest to the good quality of blood used for feeding flies and similar results were found by [33]. However, the pupal weight remains still lower than that obtained in previously studies in Cirdes insectary which was 26.98 ± 0.67 mg [32] and IBD.

From our results, the 2 sex ratios seem to show up: 1:2 and 1:10. These two ratios had better biological parameters than the other and mainly the standard ratio used in the *Gpg* normal colony. However, considering the fact that using the ratio 1:10 would necessitate more cages as the density needs to be kept at a certain level to not have a lot of mortality, and will need as well more males, it will be better to recommend the ratio 1:4. This will allow the facility to have more males for the mating or to irradiate for SIT programs.

From our results, a ratio of 1:4 could be applied to the full colonies of *G. palpalis gambiensis* (instead of 1:3), reducing female mortality and increasing colony productivity and size. Moreover, this change in sex ratio would also allow having a surplus of males therefore giving more to IBD to support the current eradication programme in Senegal [34] and for the implementation of the future SIT programs in West Africa, such as in Burkina Faso and Chad. For example, on week 16 of 2021, ~84000 *G. palpalis gambiensis* females emerged requiring 24000 males for mating with the ratio 1:3, so this change in ratio to 1:4 would save a surplus of 3000 males weekly and contribute to reaching the objective of supporting eradication programmes. In addition, the change in the sex ratio will lead to a decrease in the workload of insectarium workers since the number of females per cage will increase with the decrease in the number of males for mating, which will result in a decrease in the number of cages in colonies compared to the previous 1:3 ratio. This will result in a reduction in the number of cages of 7% in the colony and a proportional decrease in insectary workers.

5. Conclusion

The optimum male to female sex ratio should be 1:4 for *G. palpalis gambiensis* in order to enhance its longevity and productivity and also save a surplus of males.

Thus, this maximizing of the male yield would allow the IBD to meet the needs for sterile males for the eradication program in Senegal and would give more capacity for the implementation of future SIT programs in West Africa.

List of Abbreviations

AU-PATTEC = African Union-Pan African Tsetse and Trypanosomosis Eradication Campaign; AW-IPM = Area-Wide Integrated Pest Management; FAO = Food and Agricultural Organization; IAEA = International Atomic Energy Agency; IPCL = Insect Pest Control Laboratory; RH = Relative humidity; SIT = Sterile insect technique; SOPs = Standard operation procedures.

Funding

This work has been funded by *Insectarium de Bobo-Dioulasso Campagne d'Eradication de la mouche tsé-tsé et de la Trypanosomose* (*IBD-CETT*).

Acknowledgements

The authors are grateful to all the managers of IBD-CETT for the excellent working conditions and all technicians in the IBD-CETT for their contributions to the success of this work.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Murray, M., Trail, J.C.M., Davis, C.E. and Black, S.J. (1984) Genetic Resistance to African Trypanosomiasis. *Journal of Infectious Diseases*, **149**, 311-319. https://doi.org/10.1093/infdis/149.3.311

[2] Krafsur, E. (2009) Tsetse Flies: Genetics, Evolution, and Role as Vectors. *Infection, Genetics and Evolution*, **9**, 124-141. https://doi.org/10.1016/j.meegid.2008.09.010

[3] Dyer, N.A., Ravel, S., Choi, K., Darby, A.C., Causse, S., Kapitano, B., *et al.* (2011) Cryptic Diversity within the Major Trypanosomiasis Vector Glossina Fuscipes Revealed by Molecular Markers. *PLOS Neglected Tropical Diseases*, **5**, e1266. https://doi.org/10.1371/journal.pntd.0001266

[4] Solano, P., Ravel, S. and de Meeûs, T. (2010) How Can Tsetse Population Genetics Contribute to African Trypanosomiasis Control? *Trends in Parasitology*, **26**, 255-263. https://doi.org/10.1016/j.pt.2010.02.006

[5] Jordan, A.M. (1986) Trypanosomiasis Control and African Rural Development. Longman.

[6] Cuisance, D., Politzar, H., Mérot, P. and Tamboura, I. (1984) Les lâchers de mâles irradiés dans la campagne de lutte intégrée contre les glossines dans la zone pastorale de Sidéradougou (Burkina Faso). *Revue d'Elevage et de Médecine Vétérinaire des Pays Tropicaux*, **37**, 449-467.

[7] Sellin, E., Bourdoiseau, G., Clair, M., Cuisance, D., Février, J., Tazé, Y., *et al.* (1979)

Bilan de 4 années d'élevage de *Glossina palpalis gambiensis* Vanderplank 1949 (Diptera, Muscidae) à Bobo-Dioulasso (Haute-Volta), Sur animaux nourriciers (Lapins, Cobayes). *Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux*, **32**, 335-345. https://doi.org/10.19182/remvt.8124

[8] Mattioli, R., Feldmann, U., Hendrickx, G., Wint, W., Jannin, J. and Slingenbergh, J. (2004) Tsetse and Trypanosomiasis Intervention Policies Supporting Sustainable Animal-Agricultural Development. *Agriculture and Environment*, **22**, 310-314.

[9] Green, C.H. (1994) Bait Methods for Tsetse Fly Control. In: *Advances in Parasitology*, Elsevier, 229-291. https://doi.org/10.1016/s0065-308x(08)60140-2

[10] Bourn, D., Reid, R., Rogers, D.J. and Snow, J.W. (2001) Environmental Change and the Autonomous Control of Tsetse and Trypanosomosis in Sub-Saharan Africa. Environmental Research Group Oxford Limited.

[11] Vreysen, M.J.B., Saleh, K.M., Ali, M.Y., Abdulla, A.M., Zhu, Z., Juma, K.G., *et al*. (2000) *Glossina austeni* (Diptera: Glossinidae) Eradicated on the Island of Unguja, Zanzibar, Using the Sterile Insect Technique. *Journal of Economic Entomology*, **93**, 123-135. https://doi.org/10.1603/0022-0493-93.1.123

[12] Dyck, V.A., Hendrichs, J. and Robinson, A.S. (2005) Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management. Springer, 1217 p.

[13] Kabayo, J.P. (2002) Aiming to Eliminate Tsetse from Africa. *Trends in Parasitology*, **18**, 473-475. https://doi.org/10.1016/s1471-4922(02)02371-1

[14] Percoma, L., Sow, A., Pagabeleguem, S., Dicko, A.H., Serdebéogo, O., Ouédraogo, M., *et al*. (2018) Impact of an Integrated Control Campaign on Tsetse Populations in Burkina Faso. *Parasites & Vectors*, **11**, Article No. 270. https://doi.org/10.1186/s13071-017-2609-3

[15] Pagabeleguem, S., Toé, A.I., Pooda, S.H., Dera, K.M., Belem, A.S., Belem, A.M.G., *et al*. (2021) Optimizing the Feeding Frequency to Maximize the Production of Sterile Males in Tsetse Mass-Rearing Colonies. *PLOS ONE*, **16**, e0245503. https://doi.org/10.1371/journal.pone.0245503

[16] Bouyer, J., Solano, P., Cuisance, D., Itard, J., Frézil, J. and Authié, E. (2010) Trypanosomoses: Control Methods. 1936-1943. In: Lefèvre, P.-C., Blancou, J., Chermette, R. and Uilenberg, G., Eds., *Infectious and Parasitic Diseases of Livestock. General Considerations. Viral Diseases*. Lavoisier Tec et Doc, 1927-1966.

[17] Bassène, M.D., Seck, M.T., Pagabeleguem, S., Fall, A.G., Sall, B., Vreysen, M.J.B., *et al*. (2017) Competitiveness and Survival of Two Strains of Glossina Palpalis Gambiensis in an Urban Area of Senegal. *PLOS Neglected Tropical Diseases*, **11**, e0006172. https://doi.org/10.1371/journal.pntd.0006172

[18] Dicko, A.H., Lancelot, R., Seck, M.T., Guerrini, L., Sall, B., Lo, M., *et al*. (2014) Using Species Distribution Models to Optimize Vector Control in the Framework of the Tsetse Eradication Campaign in Senegal. *Proceedings of the National Academy of Sciences*, **111**, 10149-10154. https://doi.org/10.1073/pnas.1407773111

[19] Desa, G., Tsegaye, M., Lelisa, K., Argiles, R., Lema, B., Mekomnen, S., *et al*. (2018) Optimizing the Sex Ratio to Maximize the Yield of Sterile Males in Tsetse Mass-Rearing Colonies. *Academic Journal of Entomology*, **11**, 59-65.

[20] Feldmann, U. (1994) Some Quality Control Parameters Used in the Rearing of Tsetse Flies. In: Ochieng'-Odero, J.P.R. Ed., *Proceedings*: *Techniques of Insect Rearing for the Development of Integrated Pest and Vector Management Strategies*, *Volume* 1. *International Group Training Course on Techniques of Insect Rearing for the Development of Integrated Pest and Vector Management Strategies*, 16 March-3 April 1992, ICIPE, Nairobi, Kenya, ICIPE Science Press, Nairobi Kenya.

https://www.icipe.org/system/files_force/B12-%201994%20techniques%20of%20insect%20rearing%20for%20the%20develpment%20of%20integrated%20pest%20and%20vector%20management%20opz.pdf?download=1

[21] Bauer, B. and Wetzel, H. (1976) A New Membrane for Feeding *Glossina morsitans* Westw. (Diptera, Glossinidae). *Bulletin of Entomological Research*, **65**, 563-565. https://doi.org/10.1017/s0007485300006246

[22] Bauer, B., Filledier, J. and Kaboré, I. (1984) Large Scale Rearing of Tsetse Flies (Diptera, Glossinidae) in the C.R.T.A. Bobo-Dioulasso, Burkina Based on *in Vitro* Feeding Techniques. *Révue d'Élevage et de Médecine Véterinaire des Pays Tropicaux*, **37**, 9-17.

[23] FAO/IAEA (2006) Standard Operating Procedures for Mass Rearing Tsetse Flies. 239. https://www.iaea.org/sites/defaut/files/21/06/nafa-ipc-manual-tsetse_rearing_sop_web.pdf

[24] R Core Team (2019) R: A Language Environment for Statistical Computing. http://www.r-project.org/index.html

[25] Therneau, T.M.C. (2012) Mixed Effects Cox Models. 14. https://CRAN.R-project.org/package=coxme

[26] Lenth, R.H., Singmann, H., Love, J., Buerkner, P., Herve, M., Hannes, R., *et al.* (2020) Estimated Marginal Means, Aka Least-Squares Means. https://cran.r-project.org/web/packages/emmeans/emmeans.pdf

[27] Bretz, F., Hotorn, T. and Westfall, P. (2011) Multi Comparisons Using R. https://www.taylorfrancis.com/books/mono/10.1201/9781420010909/multiple-comparisons-using-frank-bretz-torsten-hothorn-peter-westfall

[28] Pollock, J.N. (1982) Manuel de lutte contre la mouche tsé-tsé: Biologie, Systématique et répartition des tsé-tsé. Vol. 1, 310 p. https://openknowledge.fao.org/bitstreams/e5713d54-0a96-4239-86fb-eed6dfe148c7/download

[29] Clutton-Brock, T. and Langley, P. (1997) Persistent Courtship Reduces Male and Female Longevity in Captive Tsetse Flies *Glossina morsitans morsitans* Westwood (Diptera: Glossinidae). *Behavioral Ecology*, **8**, 392-395. https://doi.org/10.1093/beheco/8.4.392

[30] Bonomi, A., Bassetti, F., Gabrieli, P., Beadell, J., Falchetto, M., Scolari, F., *et al.* (2011) Polyandry Is a Common Event in Wild Populations of the Tsetse Fly *Glossina fuscipes fuscipes* and May Impact Population Reduction Measures. *PLOS Neglected Tropical Diseases*, **5**, e1190. https://doi.org/10.1371/journal.pntd.0001190

[31] Abila, P.P., Kiendrebeogo, M., Mutika, G.N., Parker, A.G. and Robinson, A.S. (2003) The Effect of Age on the Mating Competitiveness of Male *Glossina fuscipes fuscipes* and *G. palpalis palpalis*. *Journal of Insect Science*, **3**, 13. https://doi.org/10.1093/jis/3.1.13

[32] Bancé, A., Kaboré, I., Sidibé, I., Rayaissé, J. and Bengaly, Z. (2012) Research Results from the Tsetse Fly Mass Rearing Laboratory at Cirdes and Their Application during 2003-2008. IAEA-TECDOC-1683. 49-62. https://www-pub.iaea.org/MTCD/Publications/PDF/TE_1683_web.pdf

[33] Toé, A.I., *et al.* (2021) 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. *Journal of Entomology and Zoology Studies*, **9**, 162-168. https://doi.org/10.22271/j.ento.2021.v9.i4b.8797

[34] Vreysen, M.J.B., Seck, M.T., Sall, B., Mbaye, A.G., Bassene, M., Fall, A.G., *et al.* (2021)

Area-Wide Integrated Management of a *Glossina palpalis gambiensis* Population from the Niayes Area of Senegal: A Review of Operational Research in Support of a Phased Conditional Approach. In: Hendrichs, J., Pereira, R. and Vreysen, M.J.B., Eds., *Area-Wide Integrated Pest Management*: *Development and Field Application*, CRC Press, 275-303. https://doi.org/10.1201/9781003169239-16