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Influence of environmental conditions on mass rearing parameters of tsetse flies at the Bobo-Dioulasso insectary (Burkina Faso): retrospective study

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ABSTRACT

With the goal of eradicating tsetse flies and trypanosomiasis in Africa, several control methods have been developed. One of this is the biological control through the application of the sterile insect technique (SIT) as a part of an area-wide integrated pest management. This method required the mass production of sterile males with a high competitiveness. The success of the tsetse mass rearing is strongly linked to the rearing conditions. The objective of this study was to do a retrospective evaluation of the impact of ambient conditions on the production performances of *Glossina palpalis gambiensis* at Insectary of Bobo Dioulasso. Data of productivity, fecundity, adult emergence and environmental conditions (temperature and relative humidity) of the rearing rooms were recorded from March to June 2020 and were analysed. The results showed a reduction of the normal pupae produced and the increase of soft pupae during this period. In addition, the relative humidity has shown a significant positive correlation with the production of soft pupae, whereas it had a significant negative correlation with the number of pupae produced per female per 10 days. However, the temperature variation during the period of the data recording didn't have any impact on the production parameters. Rearing rooms need a better management to avoid large variations in environmental parameters.

Keywords: *Glossina palpalis gambiensis*, ambient conditions, mass rearing, SIT, Burkina Faso.

INTRODUCTION

Tsetse flies are responsible for the cyclical transmission of African Animal Trypanosomiasis (AAT) or Nagana and Human African Trypanosomiasis (HAT) also called sleeping sickness. Around 50 million people and 48 million livestock are at risk of this disease (FAO, 2002). The risk is high in areas where the conditions are favourable to the establishment of the flies which need some factors such as vegetation, climate conditions, and presence of host. (Dera et al., 2022). AAT constitute one of the major constraints to the socio-economic development of sub-Saharan

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Africa because meat production is reduced by 30%, milk production by 40% and labour power can be reduced by a third (FAO, 2002). HAT is challenging to the human immune system (Oyibo et al, 2009). For the more, the cost of treatment is not insignificant because at least 25-30 million doses of trypanocides are used each year (Vreysen, 2006). To reduce the economic and health impact of HAT and HAT, several control strategies have emerged and are based mainly on parasite and vector control, but chemotherapy is confronted with the development of multidrug resistant strains of trypanosomes (Talaki et al. 2015). Nowadays, integrated pest management remains the preferred option. Integrated control of AAT involves vector control, promotion of trypanotolerant cattle, chemotherapy and chemoprophylaxis (Mamoudou et al. 2010). In view of the importance of these disease, a vast campaign of eradication was launched in July 2000 by African Heads of State and Government during the summit held in Lomé, Togo, to increase efforts to address the tsetse and trypanosomosis problem on the African continent (Kabayo, 2002). This campaign, the Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC), which involved countries in East Africa (Ethiopia, Uganda and Kenya) and West Africa (Burkina Faso, Ghana and Mali), has led to create tsetse flies and trypanosomosis-free zones. In Burkina Faso, a project in the PATTEC framework was implemented between 2006 and 2013 and 95- 99% reduction in tsetse fly densities was reached over an area of about 40 000 km^2 (Percoma et al., 2018). This density reduction should be maintained and accompanied by the application of the sterile insect technique (SIT) through the release of sterile males, taking into account the ecology and the diversity of tsetse species possibly involved in the transmission of the parasite (Zinga Koumba et al. 2014). For this purpose, a regional facility named “Insectary of Bobo-Dioulasso (IBD) was built to produce sterile male. SIT is an effective strategy to control and/or eradicate tsetse flies in an environmentally sustainable manner. This technique shown excellent results in the agropastoral area of Sideradougou between 1981 and 1984 (Cuisance et al., 1984) and on the island of Zanzibar in Tanzania from 1993 to 1994 (Vreysen et al., 2000), where eradication was even achieved. It is in this frame that the Insectary of Bobo-Dioulasso (IBD) was created to provide massively tsetse sterile males and support technically trypanosomiasis eradication campaigns in infested countries in West Africa (Toé et al., 2021). To achieve this, it is important to know the biology of the target species and master the technique of mass production and the factors that influence this production in the insectary. The IBD possesses this expertise which allow to support the project of eradication of tsetse flies in the Niayes area in Senegal (Pagabeleguem et al., 2021). The success of the Senegal project will be a guide for the future eradication projects to implement in the region such as in Burkina Faso, Ghana, Mali, Chad… and need the production of a large number of sterile males from the insectaries partners as IBD. Regarding the colony size and the needs in sterile males for the active and near future programmes, an improvement of the productivity of the colony will be necessary, as the number of pupae sent to Senegal is already lower than their request. To be able to meet these expectations, it’s important to control the different factors that can impact the production such as the ambient conditions, mainly the temperature and the relative humidity, the quality of the blood for the feeding, the hygiene in the insectary.

It was in that line that a retrospective evaluation of the impact of the ambient conditions on *G. p. gambiensis* production at IBD was done.

MATERIALS AND METHODS

Insectary

This study was conducted at the Insectary of Bobo-Dioulasso (IBD) located at Darsalamy, a village found at 15 km from Bobo-Dioulasso (11˚03'32. 4 "N and 4˚21'10.9 "W), Burkina Faso. The rearing rooms are equipped with specific materials such as air circulation, humidification and cooling to maintain specific environmental conditions for

tsetse rearing and a photoperiod of 12:12 light: dark for pupal incubation, feeding and fly monitoring**.**

Tsetse species and strain

All experiments were carried out with two strains of *G. p. gambiensis*. Cirdes and Seibersdorf. The Cirdes strain was established at Maisons-Alfort (France) in 1972 using pupae collected in the field at Guinguette, near Bobo-Dioulasso, Burkina Faso. The flies were transferred back to Burkina Faso in 1975 at the " Centre de Recherche sur les Trypanosomiases Animales" (CRTA) renamed nowadays "Centre International de Recherche-Développement sur l'Elevage en zone Subhumide" (CIRDES) (Sellin et al., 1979). In 2016, 53 972 adult flies of this Cirdes colony were transferred to IBD where a new colony was established for mass production according to the objective of the insectary and named Cirdes strain (Pagabeleguem et al., 2021).

The Seibersdorf colony was established at the Insect Pest Control Laboratory (IPCL) of the Joint FAO/IAEA Division, Seibersdorf, Austria in 2009 using pupae from the Cirdes colony. This strain, together with the other species maintained, allows member states to implement the sterile insect technique (SIT). It has also been used to support the Senegal project through the provision of sterile male pupae. In 2017, 64 213 pupae from the Seibersdorf colony were transferred to IBD where a colony was established for mass production and named Seibersdorf strain (Pagabeleguem et al., 2021).

Blood preparation and feeding regime

The adult flies were fed 4 times a week for 10 min per feeding with defibrinated bovine blood on an artificial silicone membrane in the Tsetse Production Unit. The blood was heated on a feeding plate at 36 ± 1°c (Bauer and Wetzel, 1976). This blood was collected from the slaughterhouse of Bobo-Dioulasso and Ouagadougou, with the approval of the slaughterhouse managers. After collection, the blood was immediately defibrinated and then shipped to the IBD laboratory for storage at -20˚C. After been irradiated with 1 KGry using a Cobalt 60 irradiator (Foss model 812, Sn 002) a microbial contamination test was done to assess the quality of the blood. The bacteriological test consisted of an incubation at 37˚C for 48h bacteriological culture on agarose gel in a petri dish. The quality of this blood was considered good if after these 48h, the number of bacteria colonies was less than 10. Otherwise, the blood was declared unsuitable for the feeding and discarded.

Data collection

This evaluation has been carried out from March to June 2020 at IBD. The data recorded concerned environmental parameters (temperature and relative humidity), the production of pupae (normal and soft pupae) and the emergence of the flies. The temperature and relative humidity data were recorded every 5 min using a Hobo U14-001 data logger®. In the rearing room, 9 Hobo's were installed and 4 in the emergence room. The data were extracted every week from each Hobo and served for further analysis and interpretation. Concerning the pupae production, daily collection was realized, and the total was registered at the end of the week. The soft pupae were separated, counted and discarded whereas the normal pupae were kept in an incubation room (25±1°C). This data allowed us to determine the fecundity through the number of pupae per female per 10 days (pf10d) which is a parameter used to evaluate the efficiency of the production. The emergence rate was recorded but only the emergence of the females was considered as the females are most critical gender that significantly impact the sustainability of a colony. All the parameters were recorded per strain (Cirdes and Seibersdorf).

Data analysis

Descriptive statistical analyses and correlation tests was carried out using the statistical software R Studio, version 3.2.3 (R Development Core Team 2010). Descriptive statistics were used to determine the means and standard deviations of temperature, relative humidity, pupae produced and female emergences. Student's t tests were used to

compare the means with a degree of significance $p<0.05$. The Spearman's correlation test was used to evaluate the relationship between the variables. The graphs were performed using the Excel table and the software R Studio version 3.2.3.

RESULTS

Environment parameters

Data recorded with Hobbo Data loggers® showed that in the rearing room, the mean temperature during the study period was 25.28 ± 0.44°C and the mean relative humidity was 70.96 ± 5.35%. In the pupae incubation and emergence room these parameters were respectively 25.31 ± 0.45°C and 70.65 ± 4.56% (Figure 1). The central tendency and dispersion parameters of temperature and relative humidity looked homogenous in both rooms.

The evolution per week of these parameters during the study period was up and down in the different rooms. The results showed variations from one week to another for the temperature and the relative humidity in both rooms (Figure 1 A and B). A sawtooth variation in temperature was observed. In the rearing room, the highest temperature was recorded in week 12 (26.82°C) and the lowest in week 16 (24.19°C). As for relative humidity, the general trend was an increase over time with the highest in week 27 (79.39%) and the lowest in week 13 (58.23%). The variation was significantly different for the temperature (X^2= 60.28, df = 17, p < 0.001) and the relative humidity with the time (X^2= 3657.7, df = 17, p < 0.001). In the pupae incubation room where the flies emerged, the temperature and the relative humidity varied significantly with the time (X^2= 26.542, df = 1, p < 0.001 and X^2= 90.112, df = 1, p < 0.001, respectively).

Pupae production

During the study period, 1 237 947 pupae were produced, in which 877 957 were from the Cirdes strain and 359 990 from Seibersdorf strain. The mean weekly pupae production was 45 434 and 17 904 respectively for Cirdes and Seibersdorf strains (Table 1).

The evolution of the pupae production was characterised by an instability of the production and a general downward trend in normal pupae (Figure 2A) during the study period, while an increasing trend was observed for soft pupae production (Figure 2B) of both strains of *G. p. gambiensis*. There did not exist a significant variation of the production of normal pupae according to the weeks (X^2= 7.46, df = 17, p = 0.97), however, the production of soft pupae varied slightly significantly during the study period (X^2= 27.54, df = 17, p = 0.05). The number of soft pupae was significantly lower in the weeks 16 and 17, and higher in the week 21.

The number of pupae per female per 10 days (pf10d) was recorded to estimate the fecundity of the colonies. The general trend of the pf10d during this study was down for the 2 colonies (Figure 3). There was no significant difference of the pf10d between weeks (X^2= 1.50, df = 1, p = 0.22). The pf10d of the Cirdes strain was lower (0.5961 ± 0.4422) and decreased more than that of the Seibersdorf strain (0.7406 ± 0.2351), however this difference was not significant (X^2= 64.56, df = 17, p > 0.05).

Adult emergence

As for the pupae production, the emergence of the pupae varied during the study period for the 2 colonies. However, this variation was not significantly different over the time (X^2= 14.38, df = 17, p =0.53). The means of the emergence of the females was 24 825 ± 12 111 and 12 975 ± 4 624 respectively for Cirdes and Seibersdorf. According to the male to female ratio of 1:3, the emergence allowed the introduction of 165 and 80 rearing cages (150 females x 50 males per cage) per week into each colony.

Correlation between the environmental data and production parameters

The correlation between the environmental conditions (temperature and the relative humidity) and the pupae production and the emergence of the females was evaluated (Table 2). Considering the temperature, no significant relationship has been found for the production of pupae for the two *G. p. gambiensis* strains taken separately,

either normal or soft pupae (*rho* = 0.80, *p* = 0.14). The same result was obtained for the number of pupae per female per 10 days and the emergence of the females. However, a marginal significant correlation was obtained for the total number of normal pupae (*rho* = 0.46, *p* = 0.05).

No significant correlation was obtained between the relative humidity and the normal pupae production for the two *G. p. gambiensis* strains taken separately or together. This was also the case for the soft pupae for each colony and the emergence of the females. However, a positive and significant correlation was found between the relative humidity and the total number of soft pupae (*rho* = 0.47, *p* = 0.04) (Figure 5). In addition, a negative and significant correlation existed with the pupae per female per 10 days for the 2 strains (Table 3, Figure 6).

Table 1: Summary of the data of the weekly pupae production per week.

Parameter	Strain	Minimal	Mean ± SE	Maximum
Normal pupae	Cirdes	15 320	45 434±15 139	82 530
	Seibersdorf	3 303	17 904±8 106	38 000
Soft pupae SEIB	Cirdes	370	3 341±2 364	8 262
	Seibersdorf	151	2 151±1 681	7 283
Pf10d	Cirdes	0.0400	0.5961 ± 0.4422	1.4900
	Seibersdorf	0.3000	0.7406 ± 0.2351	1.1300

Table 2: Correlation between environmental conditions and production parameters by strain.

	Strain	Temperature		Relative Humidity	
		rho	***p*-value**	***rho***	***p*-value**
Normal pupae	Cirdes	0.397	0.102	0.147	0.558
	Seibersdorf	0.176	0.483	0.143	0.569
	Total	**0.459**	**0.055**	0.261	0.295
Soft pupae	Cirdes	-0.044	0.861	0.397	0.102
	Seibersdorf	0.163	0.518	0.363	0.138
	Total	0.062	0.807	**0.474**	**0.046**
pf10d	Cirdes	0.194	0.440	**-0.824**	**<0.001**
	Seibersdorf	0.355	0.148	**-0.689**	**0.0015**
	Total	0.216	0.389	**-0.818**	**<0.001**
Emergence of females	Cirdes	-0.116	0.648	-0.073	0.772
	Seibersdorf	-0.284	0.253	0.197	0.432
	Total	-0.210	0.401	0.0361	0.886

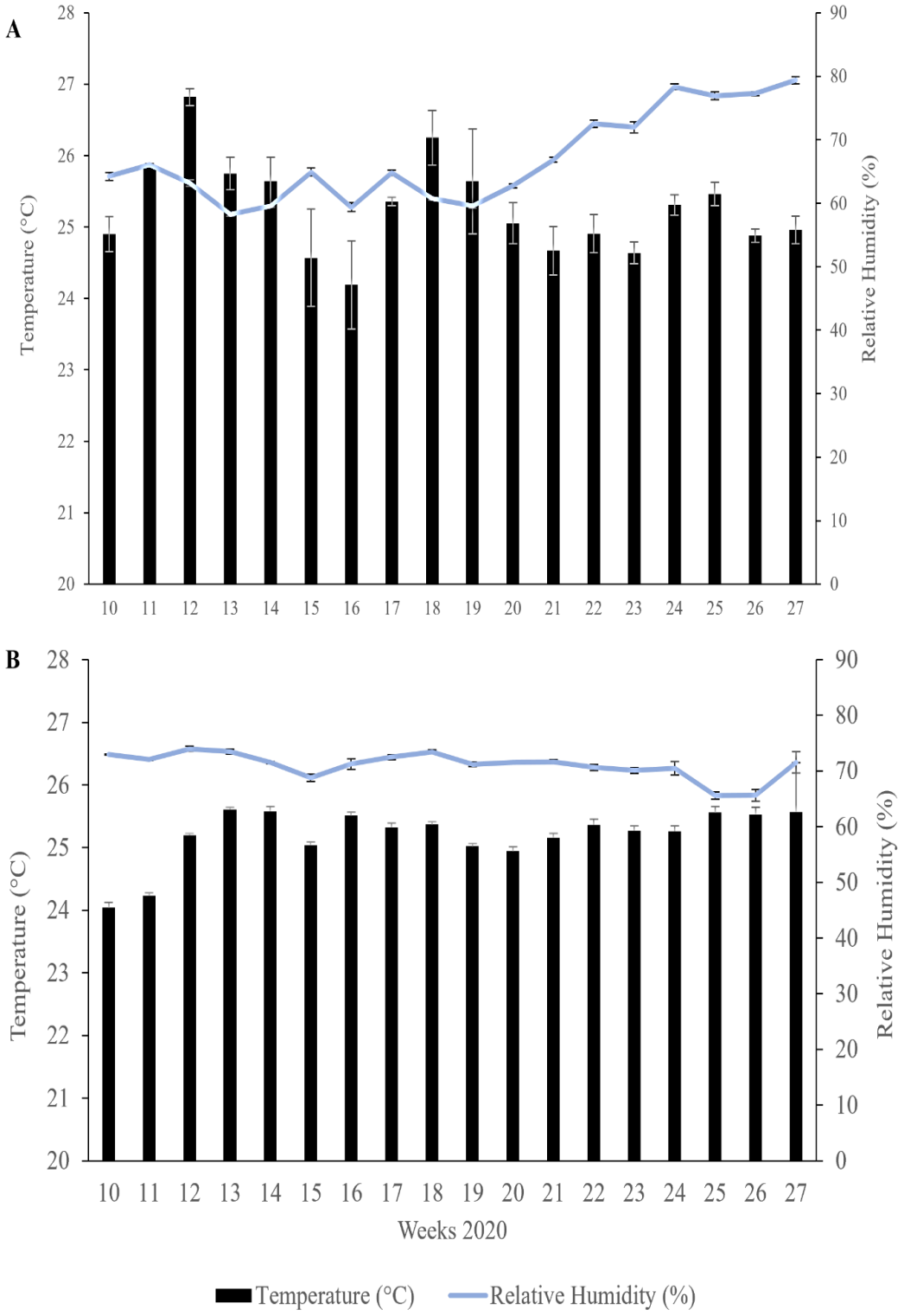

Figure 1: Variation of the temperature and the relative humidity during the study period inside the rearing room (A) and the pupae incubation room (B).

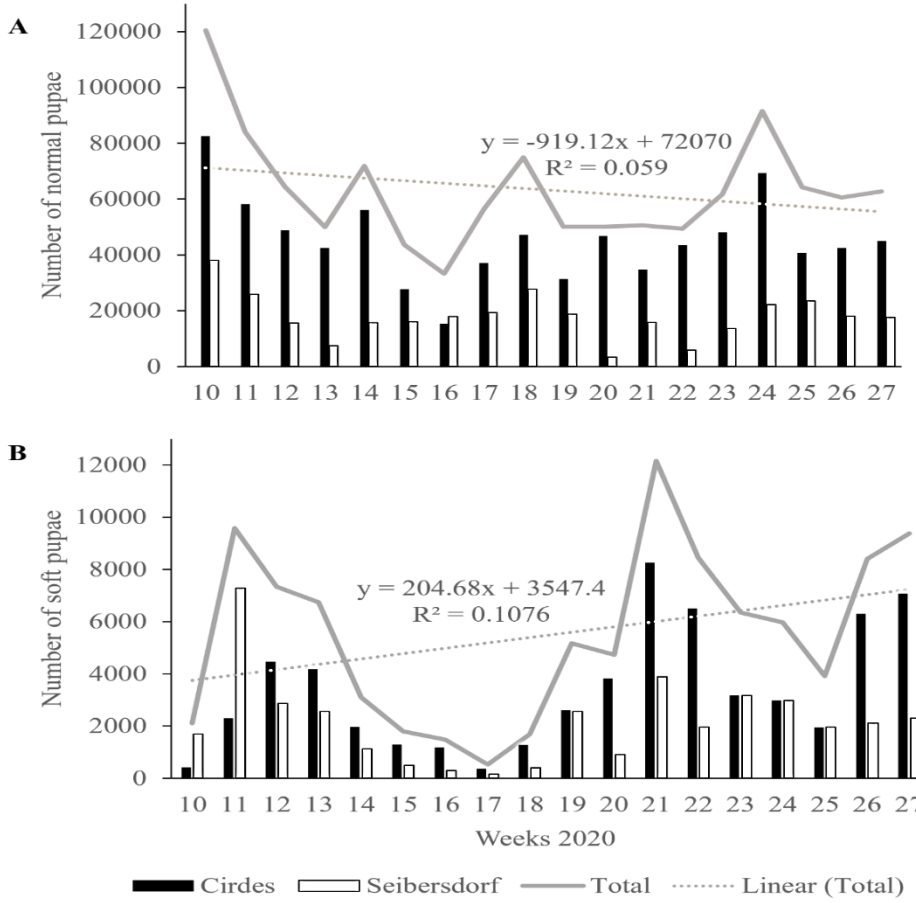

Figure 2: Evolution of the production of the normal pupae (A) and the soft pupae (B).

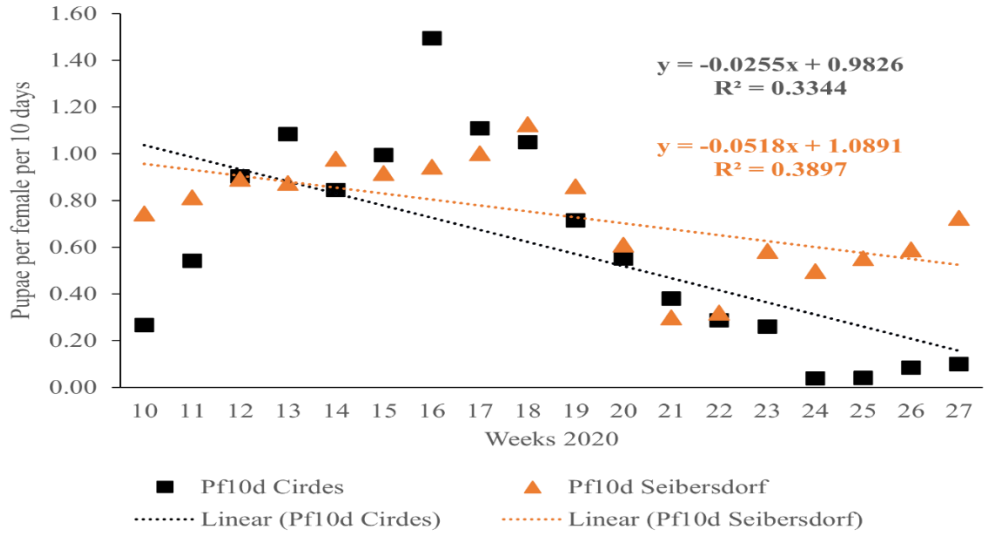

Figure 3: Evolution of the average number of pupae per female per 10 days for the Cirdes and Seibersdorf *G. p. gambiensis* strains.

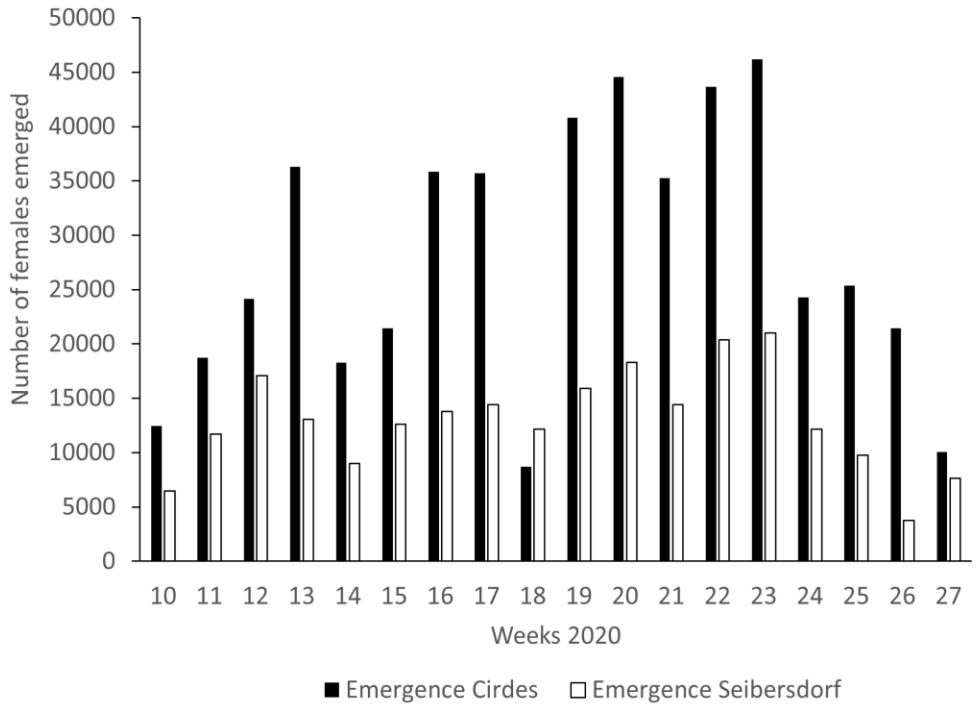

Figure 4: Evolution of the average number of females emerged for the Cirdes and the Seibersdorf strains.

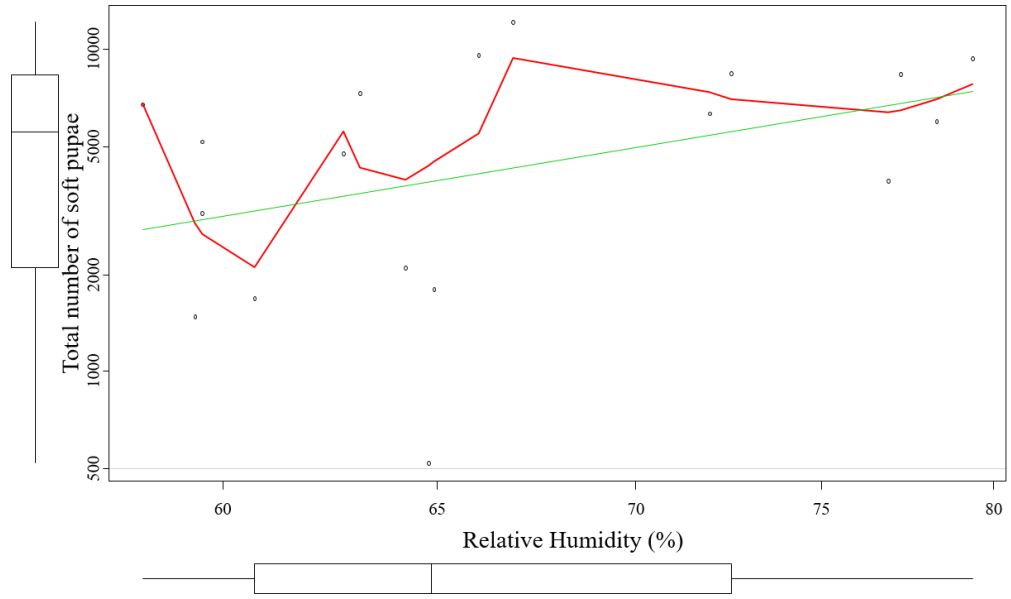

Figure 5: Correlation between the relative humidity and the production of soft pupae.
Legend: Each dot represented a value, the red line represented the evolution of the total number of pupae according to the relative humidity and the green line represented the regression between total number of soft pupae and the relative humidity.

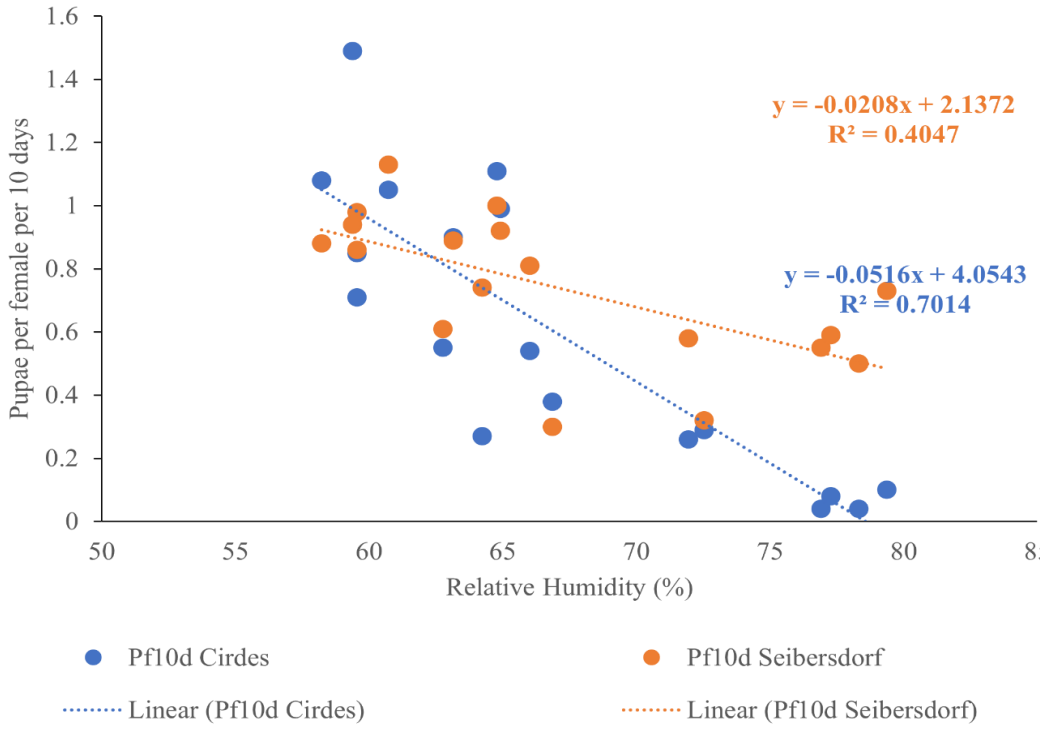

Figure 6: Correlation between the number of pupae per female per 10 days and the relative humidity by strain.

DISCUSSION

This study has been carried out to evaluate the impact of the environmental conditions on the *G. p. gambiensis* production performance at IBD. As mentioned above, the environmental conditions are one of the criteria of the success of a tsetse rearing in insectary condition and should be constant (IAEA, 1991). The parameters evaluated were the variation of the environmental conditions in the rearing rooms, the pupae incubation and the emergence of females which are very important parameters to maintain a tsetse colony.

During the study period, the average of the temperature and the relative humidity were respectively 25.28± 0.44°C and 70.96± 5.35% in the pupae production room and 25.31±0.45°C and 70.65±4.56% in the emergence room. Considering the standard conditions in tsetse *G. p. gambiensis* rearing room (temperature of 25 ± 1˚C, and relative humidity of 75 ± 5%)**,** the mean temperature was normal, but the relative humidity was slightly low for both rooms (FAO/IAEA, 2006). The slightly low relative humidity in the rooms could be due to the weak performances of the humidifiers at some time of the days. However, this result reflected the general good environmental conditions in the rearing rooms in IBD. Indeed, IBD had all the necessary equipment and materials that an insectary must have to create a favourable climate for the life of tsetse flies in the insectary, namely large capacity generators that ensure the relay in case of power cuts, a high flow borehole that ensures the supply of water. The different rearing rooms are equipped with air conditioners and humidifiers that ensure the proper conditioning of the climate in the different rooms. Digital thermo-hygrometers placed in several corners of the rearing rooms control and record the temperature and relative humidity, thus ensuring good monitoring of climatic conditions by the technicians. This

relatively good environmental conditions in IBD were recorded in previous studies in the same insectary (Toé et al., 2021). The result also illustrated the importance of maintaining equipment to ensure that, despite their depreciation, they work perfectly.

A general trend of a decrease of the production in both strains was observed during the study period. This was due to the reduction of the feeding frequency per week from 4 feeding regimes to 3. In fact, due to the restriction of Covid 19, the administration of IBD was obliged to reduce the workload by reducing the frequency of the blood collection at the slaughterhouse and the feeding frequency. This reduction of the feeding frequency then led to a decrease of the production. Previous studies on *G. p. gambiensis* (Pagabeleguem et al., 2021), on *G. pallidipes* (Mintesnot et al., 2020) and on *G. pallidipes* and *G. morsitans morsitans* (Langley and Stafford, 1990) had already demonstrated that 3 blood meals per week affected the mass rearing production (high mortality and low fecundity) than 4 or 5 blood meals per week. The number of pupae produced in the Cirdes strain represented almost the double of that of the Seibersdorf strain. This was related to the size of the colonies since the colony of the Cirdes strain is larger than that of the Seibersdorf strain. However, the pupae produce per female over 10 days which gave the best assessment of the productivity was slightly higher in the Seibersdorf strain than in the Cirdes strain. This result is similar to that of Toé et al. (2021) who found out that the Seibersdorf strain had a better productivity and survival than the Cirdes strain at IBD. The results of this study have shown an absence of significant correlation between production parameters and the temperature due to the stability of the temperature during the study period. However, the relative humidity had a negative correlation with the pupae produce per female over 10 days and a positive correlation with the production of soft pupae even though it varied between 58.23 and 79.39%. This result is different from the one of Pagabeleguem et al. (2016) which showed that a relative humidity ranging from 40 to 76% (at 25 - 26°C) had no effect on the reproduction of *G. p. gambiensis*. In these conditions, this result could be explained by the large variations between weeks rather than the range in relative humidity. The variation of the temperature during the study that didn't have impact on the pupae production was also recorded in previous studies with the same species, *G. p. gambiensis* (Pagabeleguem et al., 2016), and with other species as *G. morsitans morsitans* (Jack, 1939) and *G. fuscipes fuscipes* (Mellanby, 1936)**.** Finally, depending on the facility and the time of year, external environmental conditions may have an impact on those in the insectarium rooms.

In addition to the temperature and the relative humidity, feeding is also a factor influencing tsetse production in the insectary, mainly the quality of the blood. The blood collection and feeding protocol can have a significant influence on the maintenance and production of colonies. Indeed, the blood meal must be of good quality, containing neither pathogenic bacteria nor drug residues (antibiotics, pesticide) and the blood quality test must be more than the bacteriological, including the determination of quality factors by bioassays. According to Itard and Bauer (1984), antibiotic residues destroy intestinal and ovarian symbionts (*Wigglesworthia*, *Soladis* and *Wolbachia*), thus compromising the fertility and lifespan of female tsetse. It has been shown that a tsetse population lacking *Wigglesworthia* sterilised females but not males and succumbs rapidly to trypanosome infection (Pais et al., 2008; Wang and Aksoy, 2012; Weiss et al., 2012). These factors mentioned above need to be controlled and well monitored for successful tsetse production, in addition to the insectary internal and external environmental conditions.

Conclusion

This study was a retrospective study which allowed to evaluate the impact of the environmental conditions to the productivity parameters of *G. p. gambiensis*. The results indicated that the ambient conditions in the rearing rooms were almost stable and ranged in

the standard parameters. Only the relative humidity has impacted the productivity by reducing the pupae per female per 10 days and increasing the production of soft pupae. The study revealed the importance of maintaining constant environmental conditions and the unrespect of these parameters could impact negatively the production performances. Since the insectarium was built and equipped before the start of the mass rearing, the equipment has not been fully renewed and the absence of a highly experienced maintenance technician, the equipment would be depreciated and consequently become inefficient. To help to maintain the environmental normal, specific skills for the maintenance and repair of these materials must be acquired and implemented within the insectary. In addition, staff should keep the rearing rooms close as much as they can to avoid the exchange of temperature between the rearing room and the external environment.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHOR'S CONTRIBUTIONS

KMD, BAK and SP designed the study; BAK and AAS collected the data; KMD and BAK made the statistical analysis and wrote first draft of the manuscript; SP, AIT, MI, GMSO and AMGB made observations on the first draft of the manuscript. All the authors contributed to revise the reviewers' corrections.

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