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Post Collection Stress of Tsetse Flies Used for the Setting of Lab Colony in Mali

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Abstract: The sterile male technique (SIT), an autocidal, biological technique that respects the environment, is an efficient tool for tsetse flies eradication. This method requires many sterilized male flies released for mating with wild virgin female flies. The mass production of male flies by rearing in the laboratory and the supply of pupae is becoming more and more difficult. Obtaining sterile males in quality and quantity remains an obstacle to biological control. The aim of this study was to determine the behavior of wild tsetse flies collected in the field for rearing in the insectary to set up a laboratory colony in Mali. Collection was done in the natural habitat nearby the river in the surrounding area of Bamako by using biconical traps set from 06 a.m. to 04 p.m. the following day. Climatic and geographical parameters were recorded. Collected flies have been transferred into a cooler chamber for transportation to the insectary at the Laboratory of Entomology and Parasitology of the Faculty of Sciences and Technics (FST) of the University of Sciences, Technics and Technologies of Bamako (USTTB). Daily monitoring showed that mortality in the 72 hours post-collection varied from 10.4% to 100%. The average survival rate was estimated to 11.28% composed by 6.67% males and 4.61% females. None of them survived after 7 days in the insectary. This fact suggests that the adaptation of wild flies to the insectary needs to be further investigated to make it possible. Meanwhile, the use of pupae to set up a colony or the transfer of flies from another colony seems to be more efficient for the setting of a new colony.

Keywords: Wild Tsetse, Rearing, Stress, Adaptation Issue

1. Introduction

Since the last century to now, many studies have been done to cover numerous aspects of the *glossina* bioecology from the tsetse flies densities and geographical spread [1] to mating behavior induce by sex attractant [2] and larviposition behavior in natural conditions [3]. In Africa and especially in Mali in particular, various programs and projects for research and control of tsetse flies and trypanosomiases have been carried out. The first actions particularly targeted the main vector of HAT. Despite these advances, tsetse and trypanosomiases continue to be major health and economic problem in Sub-Saharan Africa [4].

Faced to the emergency of tsetse flies control in infested countries, there is a critical need to put together the package of efforts made by the different infested countries. The

African Heads of State and Government took the initiative to create a Pan-African program for the eradication of tsetse flies and trypanosomiases (PATTEC). This PATTEC goals to eradicate tsetse flies in Africa. The eradication strategy is innovative in itself. It is an integrated control strategy that includes a "Sterile Insect Technique" (SIT) component, based on strong research implementation.

The sterile male technique (SIT), an autocidal, biological technique that respects the environment, is an essential tool for eradicating tsetse flies. It requires numerous sterilized male flies and relies on the release of sterilized male insects to mate with wild virgin female insects. The factors conditioning tsetse rearing are closely linked to their biological and ecological characteristics. The success of the successful production of large number in the laboratory depends on the follow-up of some principles described by several authors [5-9]. The most favorable conditions are achieved with a temperature of 24 to 26°C and a relative humidity range of 60 to 85%, depending on the species. These conditions are hardly maintained in laboratories; therefore, the adaptation of tsetse flies to a new life beyond their natural habitat is difficult. The progressive orientation of insect control towards biological control requires a healthy and productive colony. Trials were carried out in different countries of Europe and Africa with the aim of studying the possibility of large numbers of tsetse flies being produced in laboratories meet to the large demand necessary for biological control by the release of sterilized male flies. From 1960 to 1970 good several of research activities were achieved with successful flies' productions in insectary [10, 11].

Some of these original colonies have, for various reasons, disappeared, and there are currently only a few laboratories where tsetse colonies are maintained without external support. CIRDES, a reference center for tsetse fly rearing in West Africa, has existed for more than 30 years. It is the only center in West Africa able to support research teams with pupae supplies. The Ethiopian insectarium similar to that of CIRDES in East Africa and The Seibeindorft Insectarium in Austria for pest control are also actives.

All these different laboratories are facing to real challenge of fly's production to satisfy the numerous demands across the world and the supply of pupae also becomes more and more difficult, which a is big limitation for biological control.

The aim of this study is to try a way of using directly wild tsetse flies to generate a laboratory colony. That requires addressing some preliminary research questions, like the understanding of wild flies population behavior in laboratory conditions in Mali.

2. Materials and Method

2.1. Study Zone

This study was carried out in the insectarium of the laboratory of Entomology and Parasitology at the Faculty of Science and Technics (FST) located on the hill of Badallabougou 12°36′58.39″N, 07°59′06.36″W. FST is a public education structure in Mali, created in 1996 with the University of Mali's current USTTB.

2.2. Type and Period of Study

This is nine month experimental and descriptive study was carried out during 9 months i.e from March to November 2020.

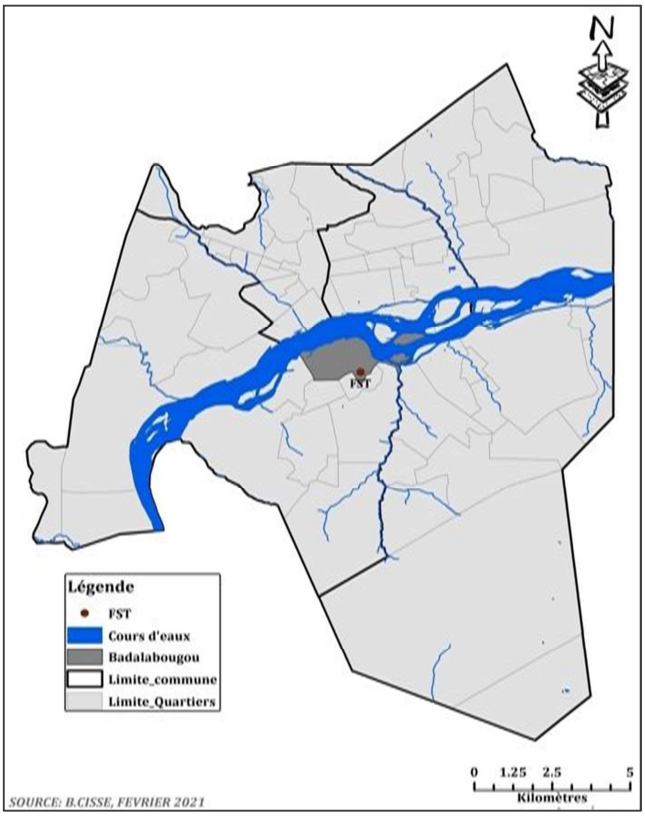

Figure 1. *Map of the study area (Source: B. CISSE, February 2021).*

2.3. Method

2.3.1. Wild Tsetse Flies Collection for Laboratory Colony Setting

Tsetse flies were collected around Kati, a peri-urban area of Bamako and transported in a cooled chamber to the insectary for the setup of the colony. To get fresh and healthy flies, traps were set along the border of the Niger river no far from vegetation. Only biconical traps were used in this work to collect active tsetse flies from the early morning (between 6 a.m. and 8 a.m.) to the afternoon of the following day (between 3 p.m. and 4 p.m.). The geographical coordinates of the traps and climatic conditions (temperature and hygrometry) were recorded. In the laboratory, all dead flies were removed, only the survivors were kept in the insectarium under optimal conditions of temperature (25 ± 1°C) and hygrometry (80 ± 5%). Live and dead flies were all morphologically identified by sex and species. For rearing, survivors were divided into cages of 60 flies according to a sex ratio of 1/3, i.e. one male for three females.

2.3.2. Tsetse Monitoring

A daily survey was carried out to record the post-setting mortality rate and the operationality rate of the flies. The dead flies were removed and recorded and the number of pupae was also recorded and set for emergence. All new emergences were recorded as well. Males and females were separated and feed with fresh blood. The monitoring and recording of temperature and relative humidity early in the morning and late at the end of the day work was regularly done. The rate of flies' operationality was determined after taking the blood meal, those that were not active were collected and checked for gonotrophic stage.

2.4. Data Analyzes

The data was entered in Excel® 2010, which was also used to perform proportion calculations and produce variation curves and histograms. Statistical tests were performed using Statistica 6.0 software. For the comparisons, the significance level was estimated at 5%.

2.5. Ethics and Biosecurity

With the approval of the veterinary office of the "Bamako Refrigerated Slaughterhouse" in district II, the blood was collected at slaughter and transported to the laboratory. The material and methods used for tsetse flies collection are harmless and have no effect on the environment.

3. Results

3.1. Collection and Monitoring of Tsetse Flies

A total of 1317 flies including 556 males and 761 females were collected. The sex – ratio was in favor of females (761/556). *Glossina palpalilis gambiensis* was the only species of tsetse found in the collection area.

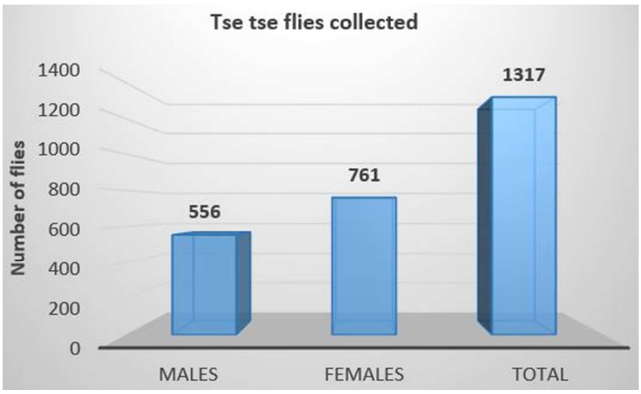

Figure 2. *Number of collected flies'.*

Post-capture stress of tsetse:

The results indicate that the mortality rate was around 70% during the 72 hours following collection. The survival rate was only around 11%. More than 18% of flies died before their first blood meal in the laboratory

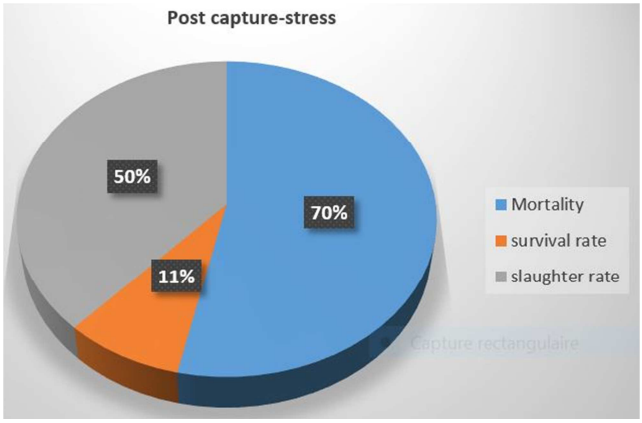

Figure 3. *Post-collection tsetse monitoring.*

3.2. Feeding Behavior of Tsetse Flies

After exposure to blood-feeding, about 25.54% of them were fed, 43.16% half-fed and 131.30 unfed. And more than half died before their first blood meal in the laboratory (Figure 4).

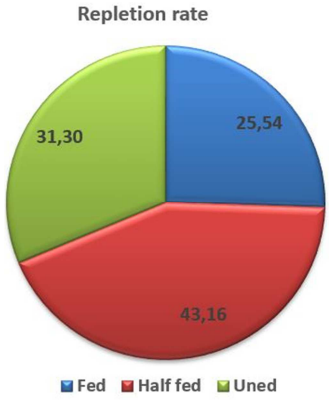

Figure 4. *Gonotrophic stages of collected wild tsetse flies.*

3.3. Mortality Observed Before the First Blood-Meal in Laboratory

About 70% of mortality was found during the 72 hours following the collection and this was before having the first blood meal in the laboratory.

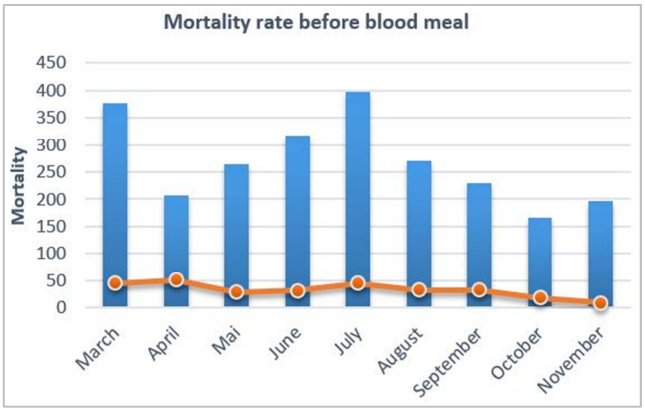

Figure 5. *Feeding behavior of tsetse flies.*

3.4. Laboratory Survey

The mortality rate varied greatly one month to another. It was high in March and July and low in October and November. The survival rate was only around 11%. (Figure 6).

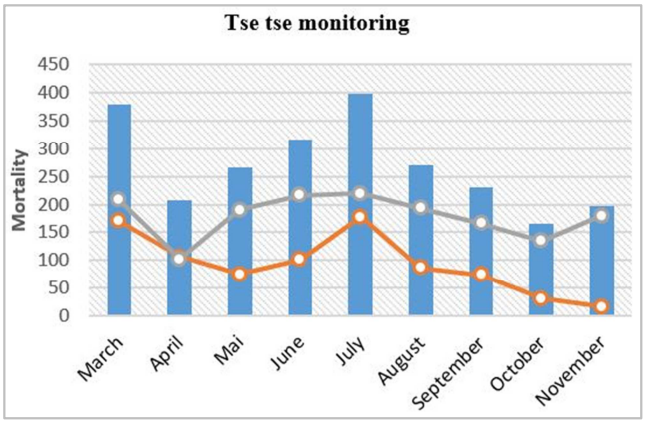

Figure 6. *Monthly tsetse monitoring.*

3.5. Climatic Conditions of the Insectarium and Mortality Rate

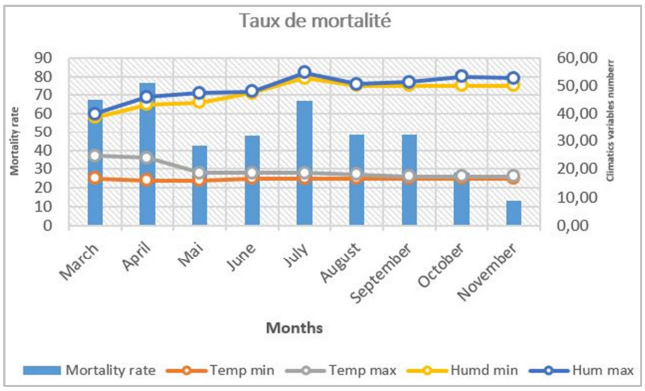

Figure 7. *Climatic conditions in the insectarium.*

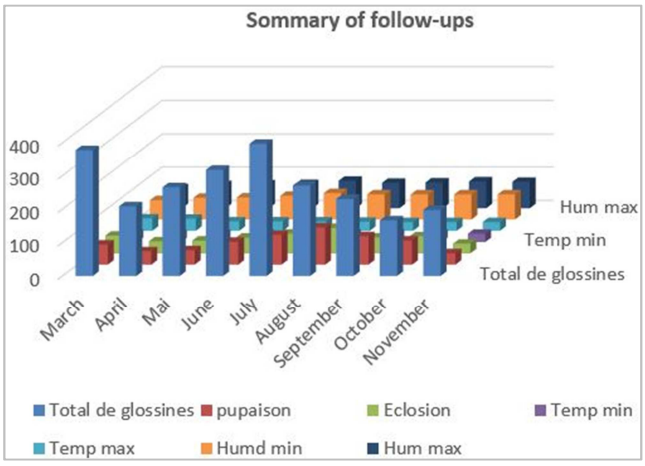

Figure 8. *Summaries of monitoring observations.*

The recommended optimum temperature for glossina rearing if 25°C, but in this experiment, the temperature was unstable and ranged between 24°C and 27°C with a maximum of 37°C. The minimum relative humidity in the insectary varied between 54 and 75% with a maximum of 82%.

4. Discussion

Wild-caught flies suffer with high mortality during the transport process and during the first days after being in the insectary. Post-collection stress is a shock that many of them cannot bear. Mortality during the 72 hours following their collection was very high. It varied between 10.4% and 100%. One of the difficulties in maintaining tsetse survival is the adaptation of wild flies to insectary conditions, and in cages flies do not have free movement compared to natural conditions. These new conditions sometime have a high cost to the flies. Therefore, flies are then highly stressed because of the change in their biotope. These factors cause a high mortality rate before their adaptation. Optimal climatic conditions are estimated to be 25 ± 1°C and 80 ± 5% for temperature and relative humidity. The mortality rate observed in the laboratory varied between 8.86% in November and 50.06% in April with an outbreak rate of 85.29% and 88.33% for the same periods. The food patterns adopted were not pleasant for many of them. This reflects a discrepancy between flies from the same group collection. Nearly 26% of the flies were starved and inactive, 43.16% half-fed and 31.30% full-fed. One reason for their inactivity could be the fact that many of them did not take laboratory blood-meals. At the insectarium, the tsetse flies are fed artificially with blood on the silicone membrane or on a foster animal, in this case rabbit was used. The average survival rate was estimated at 11.28% (267 flies) with 73 males (6.67%) against 194 females (15.25%).

The literature reports that the first tsetse breeding trials were not easy. Roubaud, was able to keep four (4) males alive for only 40 days and breeding was abandoned for lack of females [12]. In London, Mellanby reported having obtained four generations but that the colony was only slightly increasing [13].

At the Institute of Tropical Medicine in Antwerp in 1934, entomologists undertook a rearing trial which gave only bad results. [14]

McDonald asserts that there must be, in farmed flies, a lack of vitality for some, and/or the influence of sociological or ethological factors on the survival and fecundity of others [15].

F. Even reports that tsetse breeding increases only slowly since they are larviparous and only lay one larva at a time, at intervals of about 2 weeks [16]. Their life span is only a few months. This same author reports that when the flies change their biotope, according to their physiological state, it is advisable to adopt conditions presenting gradients for the various ecological factors (temperature, humidity and light), so that the flies can choose for themselves. Even the appropriate climatic conditions.

Bauer (1999), has related that the breeding would be possible only if the parental generation is, as much as possible, made up of pupae collected in the field or from wild

females captured in their roosts and raised at the same place These precautions [17]. This would considerably reduce the post-collection stresses of tsetse flies. These results confirm the successful rearing carried out in Europe by Roubaud, 1913, from pupae of *Glossina morsitans submorsitans* from Senegal for three years [12]. Strict supervision is necessary, so as to prohibit the use of any insecticide around the insectarium. One of the causes of tsetse stress and mortality could be linked to other factors with unknow sources. For example, contamination, the effects of which can be insidious and not be detected until several days after the onset of intoxication. The insecticide can be introduced into the rearing room through the clothing of staff or visitors or through equipment and cleaning products. Paint fumes, formaldehyde contained in chipboard glue or certain varnishes, tobacco smoke, and others. One cause of poisoning is a drop in the fertility rate of females, originates from certain medicinal products administered to foster animals or contained in their diet [18-20]. Therefore, the success of a rearing depends on the respect of certain principles already exposed by various authors [21-22] However, according to Bauer, tsetse flies can be kept alive for several weeks at the very site of capture by using a system of shelves on which the cages containing the flies rest.

5. Conclusion

It appears from this study that rearing tsetse from wild flies is always difficult. Their adaptation to a new biotope is a big challenge to keep them alive for more than a month. Most of the laboratory production in activity today, whether in Africa or Europe, originates from pupae collected in the field or from another laboratory. The difficulties in the rearing process could be related to ecological, climatic, environmental and feeding concerns. Sustainable supply of high quality blood meal, frequency of blood supply, achievement of climato-ecological conditions in the insectary and maintaining colonies free from infection remain challenges.

6. Recognition

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