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Large scale rearing of tsetse flies (Diptera, Glossinidae) in the C.R.T.A. Bobo-Dioulasso, Burkina based on in vitro feeding techniques

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Résumé

BAUER (B.), FILLEDIER (J.), KABORE (I.). Elevage à grande échelle de mouches tsé-tsé (Diptera, Glossinidae) basé sur des techniques d'alimentation in vitro, au C.R.T.A. de Bobo-Dioulasso, Burkina. Rev. Elev. Méd. vét. Pays trop., 1984, 37 (N° spécial) : 9-17

Des colonies de G. p. gambiensis, G. tachinoides et G. m. submorsitans ont été constituées et nourries à partir de sang de bovins recueilli sur place. Avant usage, le sang de bovins a été irradié à la dose de 50-55 krad grâce à une source de 137 Césium. L'irradiation empêche toute infection bactérienne et supprime la multiplication des trypanosomes dans la mouche tsé-tsé. L'alimentation sur lapin a été utilisée en supplément pour accroître la productivité et la taille des pupes. L'emploi de grandes cages pour le stockage de G. p. gambiensis facilite les manipulations. Une colonie de 100 000 femelles reproductrices a pu être entretenue par 5 agents techniques. D'autre améliorations techniques, telles que l'introduction de grandes plaques chauffantes et de chariots de stockage mieux conçus, permettent de faciliter le travail. Ces techniques permettent de maintenir en élevage, au C.R.T.A., environ 300 000 femelles reproductrices de glossines.

Mots clés : Elevage à grande échelle - Alimentation in vitro - Glossines - Burkina.

Summary

BAUER (B.), FILLEDIER (J.), KABORE (I.). Large scale rearing of tsetse flies (Diptera, Glossinidae) in the C.R.T.A., Bobo-Dioulasso, Burkina based on in vitro feeding techniques. Rev. Elev. Méd. vét. Pays trop., 1984, 37 (N° spécial) : 9-17

Colonies of G. p. gambiensis, G. tachinoides and G. m. submorsitans were established and fed on locally collected cattle blood. Prior to use the cattle blood was

irradiated with 50-55 krad in a 137 Cs-source. The irradiation prevented bacterial infection and abrogated trypanosomes replication in the tsetse flies. Rabbit supplements were normally given to improve productivity and offspring size. The use of large cages for G. p. gambiensis ted handling. A colony of 100 000 producing females could be handled by 5 technicians. Other technical improvements such as the introduction of large heating plates and better designed storage racks helped to reduce the work as well. Currently nearly 300 000 producing females are maintained in the C.R.T.A.

Key words : Large scale breeding - in vitro feeding - Glossina - Burkina.

INTRODUCTION

Large colonies of 3 tsetse species were established to produce sterile males for an integrated eradication campaign in a pastoral zone south of Bobo-Dioulasso, Burkina*. The area comprises approximately 3000 km^2. The 3 occuring species are : Glossina palpalis gambiensis, G. tachinoides and G. morsitans submorsitans. The tsetse colonies are fed on locally obtained cattle blood at the abattoir. The supplement of this diet by feeding on rabbit has been substantially reduced. This paper summarizes the practical experiences of an adapted feeding technology over a period of 4 years in Africa.

MATERIALS AND METHODS

The colony of G. p. gambiensis was obtained from a colony maintained on rabbits (12, 13). Initially, this colony was fed 5 days on defibrinated cattle blood and 2 days on rabbits (4). In August 1982, the feeding regimen was changed as follows: the first 4 days after emergence the flies were fed on rabbits and afterwards on heparinized** cattle blood only. G. tachinoides originating from Chad were shipped as pupae from the I.E.M.V.T., Maisons-Alfort, France. The colony in France was reared on rabbits (5). On arrival in Bobo-Dioulasso, the flies had a 5/2 feeding regimen, but since November 1982, this species has been maintained on a 6/1 regimen, i.e. 6 days on defibrinated cattle blood, 1 day rabbit supplement. The use of heparinized blood did not significantly improve the performance and was thus abandoned. Two strains of G. m. submorsitans from Burkina were introduced into the laboratory. Wild females from 2 geographically separated areas (Komoé and Samorogouan) were maintained in a specifically designed rack in the field and their pupae collected (11).

This rearing method eliminated the risk of introducing trypanosomes from wild flies into the host animals. The flies were fed on locally purchased dwarf goats. A total of 11 182 (Komoé) and 13 580 (Samorogouan) pupae were thus introduced into the laboratory. In the beginning, the majority of the emerging flies

* ex Upper Volta.

** SERVA Feinbiochemika, Heparine-Sodium, 4 I.U./ml.

were fed on rabbits, but subsequently flies emerging from 7 053 pupae (Samorogouan) were maintained on a 5/2 regimen.

The cattle blood is routinely collected 3 times per week from the local abattoir. The defibrinated or heparinized blood is stored overnight at 4°C, then 700 mg ATP and 1 000 mg glucose are added to 1 litre of blood. The irradiation of the blood is carried out in a 137 Cs-source at a dose of 50-55 krad. Previous work (3) had shown the irradiation to be an effective means for preventing bacterial infections in tsetse flies in case of a bacterial contamination of the blood. The bacteriological state of the irradiated blood is regularly controlled by placing blood samples on nutrient agar at 35° C for 48 hours. Storage of the irradiated blood is possible for at least 7 days.

Earlier described silicone membranes (2) have been slightly modified and are currently used for all tsetse species. After use membranes and silicone supports are thoroughly washed with tap water and then placed together in an oven at 100°C for 16 hours.

Presently 3 cage types are in use :

1. PVC-cages, 6 cm high, 12.5 cm diameter covered with mesh netting. These cages are used for all species.
2. ROUBAUD cages, 5 cm high, length 13.5 cm, width 8 cm for the colonies purely fed on animals and G. m. submorsitans.
3. Large rectangular cages like the ROUBAUD cages consisting of a metallic frame, 6 cm high, length 39 cm, width 19 cm which cover half of a membrane surface.

Since October 1982, these cages have been in use for a colony of G. p. gambiensis. 100 females and 30 males are permanently kept together. Laboratory equipment such as heating plates and storage racks have undergone considerable improvements and will be the subject of another publication.

The 3 species are maintained in different insectariums. Each species has at least one large production unit and a second colony for safety reasons. Insectarium, laboratory equipment and the staff are separated from the production unit. In case of an accident, the second colony can immediately serve as a backup system. Following their specific requirements, the climatic conditions are 85-95 p.100 relative humidity (r.h.) and 24-25° C for G. p. gambiensis, 70-80 p.100 r. h. and 24-25° C for G. tachinoides, 60-70 p.100 r.h. and 23-26°C for G. m. submorsitans.

RESULTS

Table 1 summarizes the performance of G. p. gambiensis. Heparinized blood was introduced for the majority of the flies during the period of observation.

TABLE I - *Glossina palpalis gambiensis*

	Mean no. of Females	Annual production of Pupae	Mean percentage of daily mortality/year
		AL 2/HAL a)	
1981	9 747	205 331	0.79
1982	14 288	231 177	0.84
1983	24 467	441 357	0.67
		AL 1/AL b)	
1981	394	5 251	1.04
1982	18 071	238 474	0.75
1983	23 910	332 112	0.72
		HAL-L.C. c)	
1983	88 219	1 003 126	0.96

a) August 1982 : heparinized blood, the first 4 days after emergence feeding on rabbits ;
b) August 1982 : defibrinated blood, the first 4 days after emergence feeding on rabbits ;
c) October 1982 : introduction of large cages, same feeding regimen as mentioned for (a).

Repeated experiments had shown the superiority of heparinized cattle blood over defibrinated cattle blood (1). All flies of this species are now fed on heparinized blood. The weekly rabbit supplement of 1 or 2 days could finally be abandoned so that from April 1983 onwards the only rabbit supplement was during the first 4 days after emergence. The introduction of large cages facilitated the handling procedures considerably. A colony of 100 000 producing females could thus be handled by 5 persons. The results of this colony in terms of longevity and productivity were inferior to the colony maintained in PVC-cages. Presently there are 150 000 producing females of this species separated in 2 colonies. The production unit comprises 100 000 females maintained in large cages, the backup colony consists of 50 000 females maintained in PVC-cages. The number of flies is deliberately kept at this level and surplus females are discarded.

Table 2 summarizes the development of G. tachinoides. The construction of a new and larger insectarium accelerated the growth of the colony numbers. In the old insectarium, there was a considerable degree of variation in temperature and relative humidity which had a negative influence on production and daily mortality. The variations could be related to the small size of the storage room and the change to a larger insectarium reduced the climatic variations to a tolerable level. The new insectarium has been in use since January 1983. At this moment, there are 2 production colonies of 45 000 and 25 000 respectively maintained in PVC-cages. Presently the feeding regimen consists of defibrinated cattle blood 6 days and 1 day rabbit supplement.

Approximately 2 years were required to adapt G. tachinoides from Burkina to the laboratory. A colony of this strain comprises 10 000 females and has the same feeding regimen as the 2 production colonies. For safety reasons, a small colony of this strain (5 000 females) is exclusively fed on rabbits. The total number of this species maintained is 90 000 females, the ultimate number will be 100 000 -

120 000 producing females.

TABLE II - *Glossina tachinoides* (Chad)

	Mean no. of Females	Annual production of Pupae	Mean percentage of daily mortality/year
		AL 2/2AL 1 a)	
1981	1 956	36 325	1.52
1982	8 623	133 999	1.61
1983	17 906	275 426	1.52
		AL 1	
1981	687	9 181	1.68
1982	3 232	50 065	1.34
1983	21 676	323 904	1.03
	Glossina tachinoides (Burkina*)		
		AL 1	
1983	2 569	1 331	0.44

a) November 1982 : rabbit supplement only once per week as for the other colonies of *G. tachinoides*

* ex. Upper Volta.

TABLE N°III-*Glossina morsitans submorsitans*

	Mean no. of Females	Annual production of Pupae	Mean percentage of daily mortality/year
		AL 2 (Komoe) a)	
1982	200	2 663	1.80
1983	3 245	14 228	0.93
		AL 2 (Samorogouan) a)	
1982	2 229	13 167	1.69
1983	12 577	48 282	1.14

a) 5 days defibrinated cattle blood, 2 days rabbit supplement.

Table 3 represents the performance of G. m. submorsitans. The initial results were characterized by a high mortality and a low rate of reproduction. This poor performance could not be related to the nature of the blood or various animal hosts. The high mortality decreased after limiting the number of females to 10 p.100 cages, but it remained high throughout 1982, and only dropped to a tolerable level in 1983. Until now the rate of reproduction has remained suboptimal, whereas the mean weight of the puparia has fluctuated between 30-33 mg which corresponds to the size of the puparia produced by G. m. morsitans. Currently 45 000 producing females of G. m. submorsitans are maintained in the C.R.T.A.: 35 000 on the mixed feeding regimen and 10 000 on rabbits. In the near future a total of at least 60 000 producing females (55 000 *in vitro*/ 5 000 on rabbits) is planned.

DISCUSSION

The system of collecting the blood in a local abattoir and its regular irradiation has now been in use for more than 4 years. Several previous experiences in the rearing of tsetse flies (2, 3, 9, 10) were characterized by bacterial infections and a subsequent increase of the mortality. The irradiation of the blood and many precautionary measures against infections during the preparation of the membrane system and the feeding prevented any outbreak of a bacterial infection. The irradiation also renders trypanosomes uninfectious to the flies since 15-20 p.100 of cattle blood from the abattoir is parasitized and yet none of the rabbits used in a mixed feeding regimen during 3 years became infected with trypanosomes.

The use of rabbits as a diet supplement for G. tachinoides and G. m. submorsitans when fed on cattle blood only can possibly be reduced by the use of porcine blood. The superiority of porcine blood over bovine blood for the maintenance of G. m. morsitans was repeatedly confirmed (7, 8). A colony of this species was maintained over years on whole defibrinated pig blood. Likewise a small colony of G. m. submorsitans is existing which is fed the same way (Maudlin, per. comm.). However the use of porcine blood as the sole source for feeding large tsetse colonies in Bobo-Dioulasso is limited by the number of pigs arriving in the abattoir, the total amount of porcine blood is approximately 100 litres per week. This is not sufficient to feed colonies of 300 000 females but it could serve as a substitute for the rabbit supplement for G. tachinoides and G. m. submorsitans.

The application of freeze-dried blood as recommended (14) for large scale rearing in Africa has considerable limitations : the performance of G. p. gambiensis fed with freeze-dried blood was inferior to the control fed with freshly collected blood (6). Furthermore freeze-dried blood cannot be offered at a competitive price.

Presently an effective means of controlling tsetse populations is the integrated campaign, that is reduction of the natural population by insecticide impregnated screens and traps and the subsequent release of sterile males. Earlier economic evaluations of the sterile insect technique (SIT) were based on time consuming and expensive methods for rearing relatively small colonies of tsetse flies. Fed on host animals for instance, it was estimated that one technician would be required to maintain 5 000 females, which means that 20 persons would be needed for a colony of 100 000 females. We have found that changes in the feeding technique from in vivo to in vitro, the employment of an advanced technology such as the use of large cages, separation of the sexes after immobilization by chilling or CO_2, large heating plates and new storage racks made it possible to handle 100 000 producing females of G. p. gambiensis by 5 persons only. Another important aspect is the degree of training for the staff. There is still place for further approaches to economize the costs of producing sterile males. However when compared with

conventional methods such as aerial application of insecticides by helicopters, the integrated campaign has already become economically advantageous. One of the most expensive factors in the SIT is the adaptation of a wild species to laboratory conditions. This adaptation can last 2 years, and it is only after this period that sufficient numbers of flies can be produced. It is therefore recommended to define areas and target species before a SIT project is launched. Other laboratories with their facilities and the expertise could then attempt to breed the target species and possibly furnish adequate numbers of an adapted species when the planned project will start.

CONCLUSION

Table 4 sums up the total number of flies species and their performance over the last year. We presently maintain nearly 300 000 producing females and this is by far the largest number of tsetse flies referred to in the literature. This also applies to the 3 principal species maintained in the insectariums of the C.R.T.A., their colony sizes are markedly bigger than any reported number of tsetse species concerned. The rearing technique described here has been in use for nearly 4 years without any major drawback and can thus be generally recommended for any project dealing with the SIT in Africa.

TABLE IV - Rearing of 3 *Glossina spp.* during 1983 in the C.R.T.A.

	Total no. of females Dec. 1983	Total annual production	Mean percentage of daily mortality
G. p. gambiensis	149 431	1 776 595	0.78
G. tachinoides	71 068	704 312	1.07
G. m. submorsitans	26 632	128 833	1.15
	247 131	2 609 740	

Resumen

BAUER (B.), FILLEDIER (J.), KABORE (I.).Cría en gran escala de moscas tse-tsé (Diptera, Glossinidae) en el CRTA, Bobo-Dioulasso, Burkina, a partir de técnicas in vitro de alimentación. Rev. Elev. Méd. vét. Pays trop., 1984, 37 (N° spécial) : 9-17

Se constituyeron poblaciones de Glossina p. gambiensis, G. tachinoides y G. m. submorsitans alimentadas con sangre de bovinos recogida en el mismo lugar. Antes del uso, se irradió la sangre de bovinos en dosis de 50-55 krad gracias al cesio. La irradiación impide cualquier infección bacteriana y suprime la multiplicación de los tripanosomas en la mosca tse-tsé. Se dió una alimentacion complementaria sobre conejo para aumentar la productividad y el tamaño de las pupas. La utilización de grandes jaulas para la conservación de G. p. gambiensis facilita las manipulaciones. Se pudo mantener una población de 100 000 hembras reproductoras con 5 agentes técnicos. Otras mejorias técnicas como la introduccion de grandes placas termógenas y de carretillas de conservación mejor concebidas permiten facilitar el trabajo. Corrientemente, se mantienen unas 300 000 hembras reproductoras en cría en el Centro de Investigaciones sobre las tripanosomiasis animales (C.R.T.A.).

Palabras claves : Cría en gran escala - Alimentación in vitro - Glossina - Burkina.

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