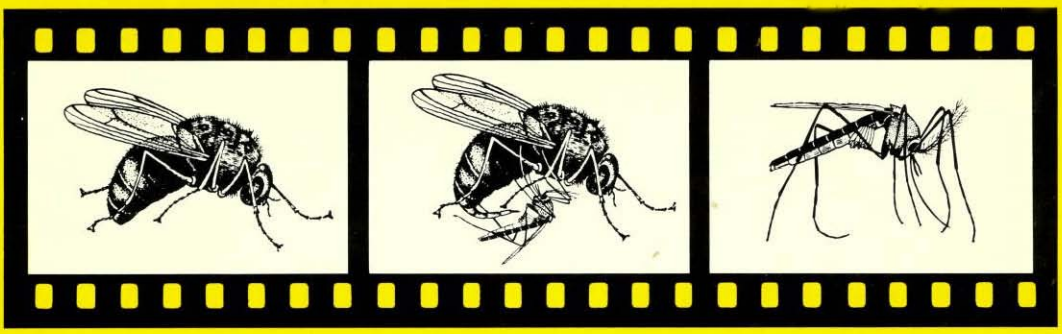

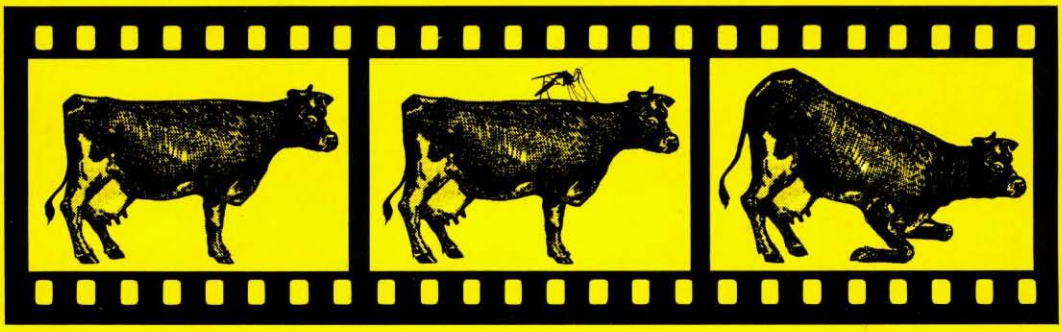

PROCEEDINGS OF A PANEL, VIENNA, 23-27 JANUARY 1967
ORGANIZED BY THE JOINT FAO/IAEA DIVISION OF ATOMIC ENERGY
IN FOOD AND AGRICULTURE

CONTROL OF LIVESTOCK INSECT PESTS BY THE STERILE-MALE TECHNIQUE

INTERNATIONAL ATOMIC ENERGY AGENCY, VIENNA, 1968

CONTROL OF LIVESTOCK INSECT PESTS BY THE STERILE-MALE TECHNIQUE

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PANEL PROCEEDINGS SERIES

CONTROL OF LIVESTOCK INSECT PESTS BY THE STERILE-MALE TECHNIQUE

PROCEEDINGS OF A PANEL
ON THE CONTROL OF LIVESTOCK INSECT PESTS
BY THE STERILE-MALE TECHNIQUE,
ORGANIZED BY THE
JOINT FAO/IAEA DIVISION OF ATOMIC ENERGY
IN FOOD AND AGRICULTURE
AND HELD IN VIENNA, 23 - 27 JANUARY 1967

INTERNATIONAL ATOMIC ENERGY AGENCY
VIENNA, 1968

CONTROL OF LIVESTOCK INSECT PESTS BY THE STERILE-MALE TECHNIQUE
(Panel Proceedings Series)

ABSTRACT. Proceedings of a panel organized by the Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture and held in Vienna, 23 - 27 January 1967. The meeting was attended by 20 scientists from 12 countries and three international organizations.

The contents include papers dealing with tsetse fly research, describing field and laboratory rearing, genetic and cytogenetic studies, effects of radiation, labelling with radioisotopes, chemosterility, and various ecological studies.

Each paper is in its original language (11 English and 7 French) and is preceded by an abstract in English with one in its original language if this is not English. The panel recommendations are also in English.

(102 pp., 16 x 24 cm, paper-bound, 16 figures) Price: US $3.00; £1.5.0
(1968)

CONTROL OF LIVESTOCK INSECT PESTS
BY THE STERILE-MALE TECHNIQUE
STI/PUB/184

FOREWORD

In 1962 the screw-worm was eradicated from the south-eastern United States by the sterile-male technique. Since that time much research has been done to determine whether this new method of insect control was applicable to other insect pests; it appears to be practical for many of the most damaging species.

A Panel concerned with the control of livestock insect pests was held by the Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture from 23 to 27 January 1967 at the Headquarters of the International Atomic Energy Agency in Vienna. Twenty scientists from 12 countries and three international organizations attended. The Panel recognized the need for wider communication in livestock pest research and recommended the establishment of a co-ordinated programme of research on the use of radiation to control animal insect pests, with an emphasis on genetic aspects.

The programme, which is in progress of being initiated, aims to establish a mechanism for international co-ordination of the work of some of the more active research workers in radiation control and eradication of insect pests to animals, and to accelerate the improvement of methods of inducing genic or chromosomal mutations.

The Panel dealt mainly with the possibilities of using the sterile-male technique to control various livestock pests, including torsalo and tsetse flies. A large number of the 18 papers concerned tsetse fly research, describing field and laboratory rearing, genetic and cytogenetic studies, effects of radiation, labelling with radioisotopes, chemosterility, and various ecological studies. It was stressed that detailed knowledge in genetics, ecology and mass rearing are pre-requisites to any sterile-male technique. The kinds of knowledge required and specific problems are outlined in the Recommendations.

It is hoped that the publication of these papers will stimulate the research that is essential for successful applications of the technique. Another book in the IAEA Panel Proceedings Series, entitled "Radiation, Radioisotopes and Rearing Methods in the Control of Insect Pests", has been published recently.

CONTENTS

REPORTS

POSSIBILITIES FOR USING THE STERILE-MALE TECHNIQUE IN TORSALO, Dermatobia hominis, AND OTHER LIVESTOCK PESTS IN THE OIRSA AREA

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Abstract

POSSIBILITIES FOR USING THE STERILE-MALE TECHNIQUE IN TORSALO, Dermatobia hominis, AND OTHER LIVESTOCK PESTS IN THE OIRSA AREA. Ecological and other biological studies have been investigated for torsalo with a view to initiating a programme of control by the sterile-male technique. Basic radiobiological information is available; however, artificial rearing continues to be unsuccessful.

INTRODUCTION

The success of the sterile-male method of eradicating the screw-worm, Callitroga hominivorax (Cqrl), from Curaçao and the south-eastern United States of America (Bushland 1960) suggested the possibilities of utilizing sexually sterile insects for controlling or eradicating other species (Knipling 1955 and 1964), and several workers have pointed out that this might be a feasible way to control and eradicate torsalo.

The use of an organized control measure was recognized by the ministers of agriculture of Panama and Mexico at the XIth CIRSA Meeting held in 1963. OIRSA (Organismo Internacional Regional de Sanidad Agropecuaria) was authorized to organize and initiate a research programme in co-operation with the Ministry of Natural Resources of Honduras. The initial studies for establishing such a research project were supported by the livestock insect investigation laboratory of the US Department of Agriculture at Kerrville, Texas, and by the FAO through their regional veterinarian for Central America, Mexico and Panama.

ECONOMIC IMPORTANCE

Dermatobia hominis cause extensive damage to the cattle industry in tropical Latin America. Torsalo (D. hominis) infest a number of wild animals, and livestock is considered the most important host for this

species, and frequently humans are also attacked. Yearly losses in the livestock industry caused by D. hominis are estimated for Honduras alone at approximately US $1.8 million. Andersen (1962) has estimated a yearly loss of livestock at US $4 million for the Central American countries.

The parasitic stage of the insect is the larvae that bore into the skin of livestock and lodge themselves in the subcutaneous tissue where they feed for about 45 days. Moving animals to market from infested areas to non-infested areas over the years has contributed to the distribution of the species into new areas.

When the chlorinated insecticides such as toxaphene and BHC became available they were used to treat infested animals to control torsalo; however, they were not effective against larvae that had established themselves in the animal. For this reason effective control was not obtained.

When the new systemic insecticides came into use, a high degree of control was obtained. However, it seemed unlikely that large-scale eradication could be achieved with an insecticide programme alone, because of such factors as the high cost of the insecticide and management practices and the terrain in many localities being unfavourable to an insecticide programme at a national level.

FEASIBILITY OF APPLYING THE STERILE-MALE TECHNIQUE

After estimating the basic information needed to determine the feasibility of applying the sterile-male technique to control and eradicate D. hominis, OIRSA established in 1963 a research programme to obtain the necessary knowledge of the biology, ecology, natural and artificial rearing of the larvae, the feasibility of establishing a laboratory colony and sterilizing the insect by irradiation. At present the requirements listed in Knipling (1964) for a pilot test release programme seem to be fulfilled.

During the three years study we have obtained much information relating to the life of Dermatobia, including: duration of the life stages were studied, and bovines, rabbits, guinea pigs and goats were artificially infested for rearing the larvae. A way of artificially rearing the larvae to maturity on diets has not yet been found, but encouraging results have been obtained. Temperature, relative humidity and other optimal conditions for rearing the insects under laboratory conditions have been achieved, and consequently a colony has been established that still uses the natural host to rear the larvae. Studies on the external and internal anatomy have been completed. Tests showed that no practical difficulties exist regarding sterilization of this species by means of ionizing radiation.

A field station was established to provide information on the natural habits and field infestations of the species and much useful data was obtained.

INTEGRATED CONTROL AND ERADICATION PROGRAMME

Studies related to the habits of this insect showed that the female fly only occasionally comes into contact with the host animal, and that

the adult female Dermatobia fly lays her eggs on other flies that serve as vectors. It seems that an integrated eradication programme could be applied beneficially in conjunction with a systemic insecticide to decrease natural populations of Dermatobia before releasing sterile insects, and afterwards both methods could be combined.

OTHER LIVESTOCK PESTS

Based on the results obtained in the south-eastern United States of America from the use of the sterile-male technique for eradicating the screw-worm fly, this programme has been extended to the northern states of Mexico with the hope of eventually eradicating the insect from Mexico. Considering that the screw-worm is an important livestock pest in Central America and that there are good possibilities to extend further this programme of eradication to these countries, OIRSA has begun to evaluate infestations and to accumulate information on the screw-worm situation in Guatemala. This work is being carried out in co-operation with the Guatemalan Ministry of Agriculture. Training programmes have been conducted to prepare technical personnel. Considering the great economic importance of ticks in the livestock industry, a survey was initiated to classify the Ixodidae in the OIRSA region in the hope that a way may be found to eradicate these by means of the sterile-male technique.

PROSPECTS OFFERED BY THE LABORATORY BREEDING OF Glossina morsitans IN LISBON*

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Abstract

PROSPECTS OFFERED BY THE LABORATORY BREEDING OF Glossina morsitans IN LISBON. For several years Glossina morsitans collected in Rhodesia and Mozambique have been successfully reared in the laboratory. Rearing methods, an insecticide accident and various biological aspects of tsetse are discussed. Radioisotope techniques for labelling studies are described.

1. INTRODUCTION

The first attempt at breeding, or at least at maintaining, the tsetse fly in the laboratory was made as early as 1895, when Bruce discovered the transmission of nagana trypanosomes by Glossina pallidipes. The problem, however, was finally solved only in 1959, with the successful maintenance in the Institute of Tropical Medicine of Lisbon (Department of Entomology and Helminthology) of a colony of G. morsitans morsitans as an autonomous population. More recent work on the subject is Nash's promising work on the breeding of G. austeni [1], and since 1964 Itard and Maillot [2] have been maintaining two G. morsitans colonies with considerable success (a maximum of eight generations in one colony up to 1966), as well as one G. tachinoides colony with less good results. However, more time is needed before any definite conclusions may be drawn from the results obtained.

Meanwhile, several unsuccessful attempts have been made, both in Africa (an adequate climatic environment for tsetse life) and in Europe (in climatized laboratories), to breed these flies.

According to the results obtained so far in Lisbon, the first of which were presented in 1964 to the XIIth International Congress of Entomology [3], we believe we have managed to solve the problem of the laboratory breeding of G. morsitans.

2. MAINTENANCE AND EVOLUTION OF THE GLOSSINA POPULATION IN LISBON

Up to now we have been maintaining our glossina colony by employing the technique summarily described in the World Health Organisation Bulletin of 1964 [4].

* This work was subsidized by the Portuguese Overseas Research Board (Junta de Investigações do Ultramar, Lisbon), World Health Organisation, Department of Agriculture of the United States of America, Companhia de Diamantes de Angola, Instituto de Alta Cultura (Lisbon), the Calouste Gulbenkian Foundation and the Portuguese Nuclear Energy Commission (Junta de Energia Nuclear, Lisbon).

The details of the method were fundamentally as follows:

(1) The colony was permanently kept inside a climatized chamber at a temperature of 26°C and with a relative humidity of 55-70%.
(2) The breeding chamber had fluorescent lighting (510 lx) from 6.00 to 18.00 hours.
(3) The females were permanently isolated in net Roubaud cages, except when mating; one male was put with one female for two to three days.
(4) The flies fed on guinea-pigs supplied daily for 30 min; care was taken to make sure that every glossina fed.
(5) The pupae were placed in damp sand as soon as possible after their formation until the adult eclosed.

Among the factors that occasionally hindered the normal growth of our colony, temperature was the most relevant. In the summer of 1964, when the temperature inside the insectaria rose to 30-32°C for two hours due to faulty controls, 20% of the colony was lost.

From 1959 to November 1963, all the details of the technique described above were followed with the exception of the item (5); once the pupae were formed they were kept in ordinary glass tubes. Only after damp sand was used for installing the pupae did the colony show a remarkable growth rate.

Two distinct phases in the evolution of the colony are therefore apparent: an adaptation phase in which the population presented a hesitating and occasionally even precarious advance, corresponding to the first 17 generations, and a growing and stabilized phase beginning in November 1963. The colony is designated the standard colony and it is now in the 36th generation.

The only change introduced in the technique that had been followed up to 1963 was the use of damp sand for keeping the pupae, so we assumed that this was the reason for the progress of the colony.

However, as we wanted to assess the influence of the damp sand on the favourable growth of the glossina population, we studied the evolution of an already stabilized populational sample of pupae placed both in common tubes, as before, and in damp sand. We noticed that the pupal eclosion rates were identical in two lots of 500 pupae. We therefore concluded, contrary to what we had previously supposed, that the damp sand was not the determining factor in the progress of our colony, although it is not yet certain whether the final evolution of the population from the pupae maintained in the tubes is as good as the population from the pupae maintained in damp sand. Meanwhile, we must also consider the hypothesis discussed by Willet [5] to the effect that genetic selection might produce a generation adapted to life in confinement.

We believe that to explain the good results obtained we must not omit other hypotheses, as we still do not know the factors that are really necessary for the natural reproduction of glossina, whether in confined areas or in bigger spaces. MacDonald [6] has reported that G. morsitans submorsitans breeds better in large cages than in individual tubes.

Meanwhile, by employing the technique described on a new G. morsitans population initiated in July 1966 from some 1800 pupae kindly sent to us

from Rhodesia by Dr. D. A. Dame (Rhodesian colony), we noticed that the behaviour of the new population agreed with the adaptation phase of our colony (Table I). We expect a stabilized phase to follow the adaptation phase, as in our standard colony.

Besides maintaining the already mentioned G. morsitans populations, in April 1965 (R. C. Pinhão), under precisely the same conditions we initiated a G. submorsitans population from some pupae kindly sent from Nigeria (West African Institute for Trypanosomiasis Research).

The evolution of this colony has progressed in the same way as the standard colony had done; the colony was begun with seven females and ten males and has now reached the 9th generation, with 67 females and 20 males.

We may conclude that the breeding technique we have adopted solves the problem of large-scale culture of G. morsitans. If our population had been totally maintained from the beginning of the ascending phase, at the end of 1966 we should have had a total of 10 447 360 adults, a considerable number that would permit a large amount of research into glossina, particularly concerning the utilization of sterilized males to control these insects.

It may be interesting to report a serious accident that occurred in 1965 during the evolution of the colony and that could well have been fatal to it. Without our knowledge, a commercial insecticide with a dieldrin base was applied to the walls of the compartment where we kept the guinea-pigs on which the glossina fed. The amount of insecticide that adhered to the guinea-pigs' fur must have been minimal, since the animals had been removed from the enclosure before the insecticide was applied, and were put back in it only after the walls and floor had been thoroughly washed. Nevertheless, the amount of insecticide was enough for us to lose in two days 623 specimens (508 males and 105 females) of the 2216 adults.

Apart from this immediate mortality, which mainly affected the males (Table II), there was also some delayed mortality (Fig. 1) as the population remained affected for six months, as was shown by the following signs: early death of adults; decrease of the pupal eclosion rate (Fig. 2); the adults eclosed from these pupae refused to feed, in spite of all the adequate precautions such as replacement of guinea-pigs, cages and feeders, etc.

TABLE I. COMPARISON OF THE INITIAL DEVELOPMENT OF THE FEMALES OF THE STANDARD AND RHODESIAN COLONIES OF G. morsitans

	Monthly variation in population	
Month	Standard colony	Rhodesian colony
2	1.3	0.8
3	1.5	1.3
4	1.2	1.2
5	1.1	1.1
6	0.8	1.1
Stabilized phase	1.5	

TABLE II. UNEXPECTED MORTALITY OF THE COLONY DUE TO INSECTICIDE (1965)

Daily mortality	Days of month	Females		Males	
Normal		Total	Percentage	Total	Percentage
			0.5-1		3-4
After insecticide	26	55	3.6	272	38.8
July	27	60	4.0	236	52.6
	28	29	2.3	68	29.3
	29	27	1.9	49	27.5
	30	25	1.8	26	19.0
	31	14	1.0	19	14.0
	Total (26-31 July)	210	2.5 (Mean daily)	570	33.4 (Mean daily)
August		1004	3.2	293	5.6

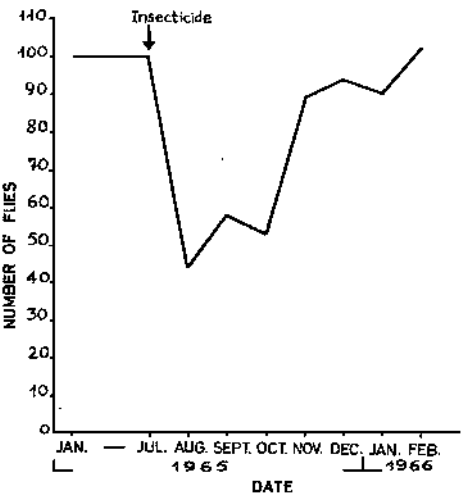

FIG. 1. Evolution of the colony after the insecticide accident (number of flies on the last day of the month).

Because of this accident, we lost the generation that was directly affected by the insecticide. The colony was saved by the pupae that were evolving (Fig. 3) and which were not affected by the drug. Thus, from the minimum number of 200 specimens in January 1966, we have progressed to the present figure of 1200 (10 January 1967); this amount, plus a similar number of the Rhodesian colony, form a total of about 2400 flies, entailing an arduous task in maintenance and recording.

The insecticide accident, which, however, did not destroy our colony, confirmed that our technique is satisfactory, and that the colony is well adapted to the laboratory since it has been growing without any signs of degeneration.

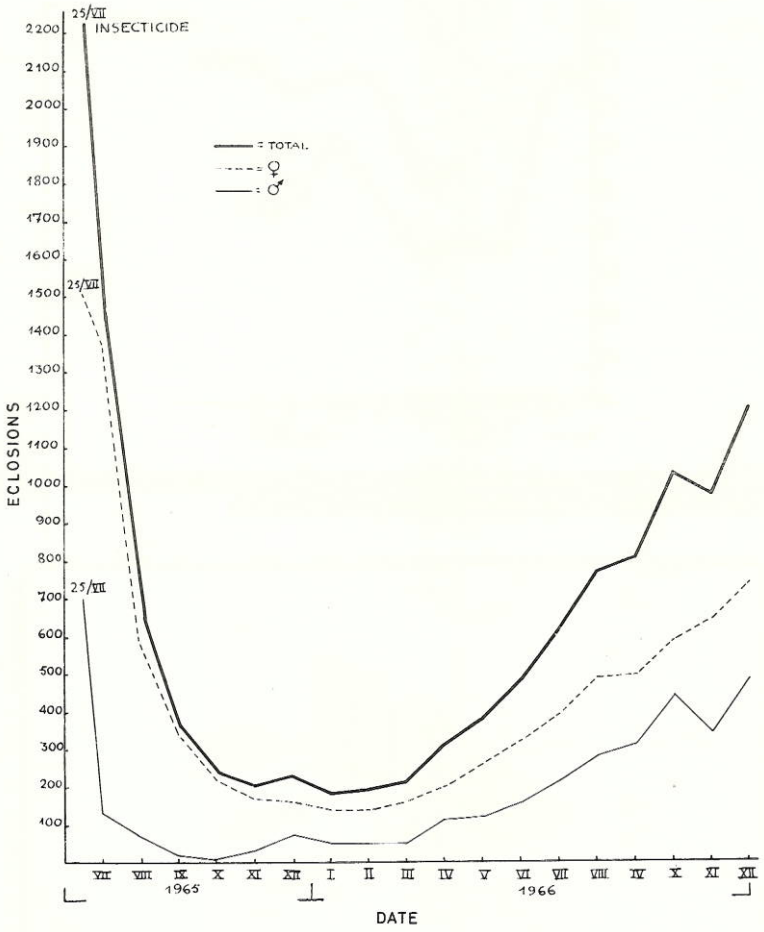

FIG. 2. Rates of eclosions of the colony after the insecticide accident.

We must, however, report some inconveniences in our method, such as the large number of auxiliary staff needed to ensure the daily feeding of all the specimens, and the considerable space needed for the Roubaud cages. When the colony reached 2216 adults, we were using two rooms of 23 m^3 each, which were already crowded; if the colony increases at an average monthly rate of 1.5%, it will be very difficult to maintain it.

Regarding the large number of staff required, it will be difficult, if not impossible, to reduce the number under present conditions, but we are planning to use larger animals such as rabbits [1] for feeding, or to reduce the number of feedings.

We are trying to solve the space problem by using small net cages (Fig. 4), of 8 cm × 4 cm × 4 cm (volume 128 cm^3), which are about one-third the size of the Roubaud cages (volume 448 cm^3). We do not know

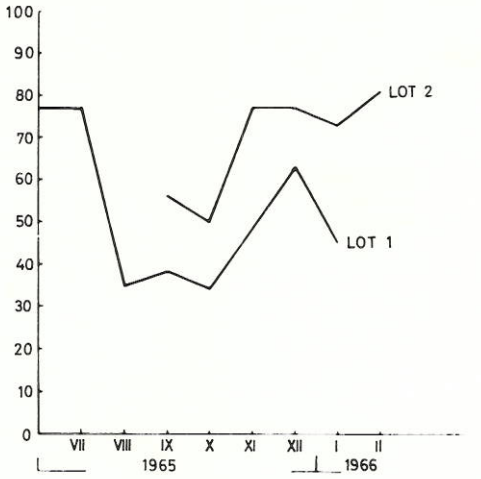

FIG. 3. Rates of eclosions of pupae descending from females that suffered from the "direct" action of the insecticide (Lot 1) and those of the pupae born after the accident (Lot 2).

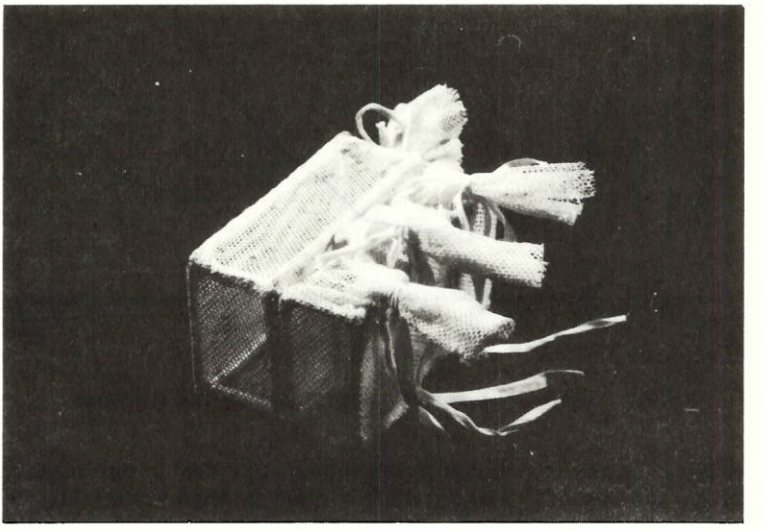

FIG. 4. Comparison of the "minicage" and the Roubaud cage.

to what extent the life of the glossina will be affected by such a small confined space but instead of one cage at a time we can use three such cages at the same time in which the flies can feed on the guinea-pigs. If this "minicage" is successful, we shall have mainly solved the problem

of the space necessary for the maintenance and feeding of the colony. In addition, we shall place the pupae directly in these cages instead of transferring them to the tubes, thus avoiding handling with consequent saving of time and space. As this new technique was tried only a short time ago, we still lack adequate experience; we hope that it will prove satisfactory and that it will simplify our breeding method. For the time being, we are placing the pupae evolved each day in the same tube to solve the space problem.

3. UTILIZATION OF THE GLOSSINA POPULATION[1]

We have specimens of different ages as well as individuals of both sexes in enough quantities for experiments. We willingly place our colony at the disposal of any interested institutions. The University of Edinburgh (Drs. Lumsden, Saunders and Mews) and the Institute of Tropical Medicine of Anvers (Professor Jadin and Drs. Olyslager and Evens) are trying to form their own colonies (sub-colonies) initiated from pupae we have sent them from our standard colony. We are also meeting requests from London (Professor D.S. Bertram), Tanzania (Dr. N.S. Irving), Kenya (Dr. T. Odhiambo), Canada (Dr. K.C. Davey), the United States of America (Dr. Axtell), and elsewhere.

Meanwhile, we were able to study some aspects of certain biological details of the glossina in our colony, and a detailed file-card has been made for each specimen containing the following data:

Females: origin; date of larviposition; date of death; date of eclosion; pupal period; larviposition; descendants; longevity; abortions; interval of the first larviposition; average interlarval period; interval between larvipositions; number of generations; date. Males: origin; date of larviposition; date of eclosion; date of the death; pupal period; longevity; number of generations:

Our colony was also used to label glossina with radioisotopes, and to study their microbial flora.

The results of the study of the sexual aggressiveness of males bred in captivity in comparison with the wild ones, another subject for which our colony was used, is reported by Dr. Dame[2].

3.1. Biological data

Some evolutionary aspects concern life expectancy, eclosion rates, the duration of the pupal period, the interlarval period, fecundity, sterility, sex ratio and causes of early death.

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[1] For more detailed studies on this subject see the doctoral thesis of Dr. R.C. Pinhão (to be published).

[2] DAME, D.A., "The present situation and future prospects for control of tsetse by the sterile-male technique", these Proceedings.

3.1.1. Life expectancy

A progressive increase in the average life expectancy was observed in the course of adaptation to the laboratory. Average life expectancy figures of about 90-100 days for females and of approximately half these for males were considered normal. The individual maximums were 246 days for one female and 166 days for one male. The average life expectancy of our glossina has progressively decreased until the 7th generation, was constant though fluctuating in the following generations, and began to increase after the 17th generation; the figures observed after the 20th generation were above those reported for the flies of the 1st generation.

3.1.2. Eclosion rates

During the first eight months of our colony, the average value of the eclosion rates was 95%. Thereafter they showed a tendency to decrease and were at an average of 86% in October 1963. Then there was a rise in the average value, then attributed to the adoption of the technique of keeping the pupae in damp sand; the new monthly eclosion rate was about 90%. In mid-1964 the eclosion rates showed a new tendency to decrease, which we attributed to the deficient feeding of the females as the large size of our population at that time did not permit the usual care. In 1966 the eclosion rates ranged from 82 to 90%.

3.1.3. Duration of the pupal period

We confirmed that the pupal period was consistently shorter in females than in males, but we noticed that the difference tended to increase as the temperature at which the pupae were kept diverged from an average of about 27°C. At this temperature, the average pupal periods of G. morsitans were 26 and 24 days for males and females respectively.

3.1.4. Interlarval periods

The average interlarval periods lasted for about 11 days at 26°C and remained constant with no tendency to increase with the aging of the females.

3.1.5. Fecundity

Fecundity depended on longevity and thus the number of larvae per female followed the life expectancy. In the first 20 generations we obtained an average of 5.3 larvae per fertile female, with a maximum of 8.3 and a minimum of 2.8. A maximum of 20 larvae was observed in one female.

3.1.6. Sterility

The rate of fertile females was 83.7%, which is higher than in previously reported results. It was noticed that the age of the females when coupled affected the sterility rates considerably. Contrary to what is sometimes recommended in literature, it is preferable to mate the females early.

3.1.7. Sex ratio

The sex ratio has remained constant in our population, with eclosion of practically equal amounts of males and females.

3.1.8. Causes of early death

We do not know the reasons for the early death of some of our glossina. As a rule, death occurs after a period of some days during which the flies do not feed spontaneously. We therefore consider this a sign of debility, and it is possible that early death may be mainly related to a congenital debility.

3.2. The radioisotope labelling of glossina

This part of our work, performed with the co-operation of Drs. Carvão Gomes and Bragança Gil of the Laboratory of Radioisotope Studies of the Overseas Research Board, had two objectives: (a) To assess the possibility of labelling the glossina for studying their populations in nature; (b) To attempt to label the spermatozoa for studying the possible cross-breedings between species or varieties through the radioactivity observed later on in the spermathecas.

3.2.1. Technique for the labelling of glossina

We believe that the best way of labelling the glossina would be to administer the radioisotope in their blood feeding and, accordingly, we used phosphorus as this element is a universal metabolite. Jenkins [7] in his synthesis on isotopes applied to entomological observations quotes ^{32}P and ^{90}Sr as being the isotopes most used for studying insect ecology. ^{90}Sr was eliminated for safety reasons. Meanwhile we plan to use this radioisotope in future taking all precautions because of the suitability of its application on account of its long half-life compared with that of ^{32}P.

Up to the present we have made two tests, using in the first a male guinea-pig weighing approximately 670 g and intraperitoneally injected with labelled ortophosphate solution with an activity of 220 μCi, corresponding to a dosage of 0.343 μCi/g; in the second test the dosage was increased to 1 μCi/g and we used a male guinea-pig weighing 780 g, injected with an activity of 780 μCi.

The radioactivity of the guinea-pigs' blood was measured by a window-type Geiger-Müller counter using a blood volume of 0.01 ml collected from the animals' ears. Radioactivity readings of the blood samples from the guinea-pigs are shown in Tables III and IV.

The activity achieved by this method was weak and therefore it will be difficult to obtain considerable dosages in glossina fed on the guinea-pigs' blood, but we intend to increase the activity of the radioisotope to be injected in other experiments. In spite of this, the glossina were fed on successive days on the guinea-pigs labelled with ^{32}P. After each feeding and before the next one, the activity of each glossina was counted. The insect was placed in a glass tube (diameter 1.5 cm, height 4.5 cm) whose opening was then covered with a piece of gauze. The insect alights on the gauze and the tube is introduced into a lead shield-holding device that fixes its

TABLE III. ACTIVITIES OF THE SAMPLES OF BLOOD OF GUINEA-PIG 1

Date	Volume of blood (ml)	Average activity (in counts/min)
7 December 1966	0.01	34
9 December 1966	0.01	36
10 December 1966	0.01	32
12 December 1966	0.01	36
13 December 1966	0.01	40
14 December 1966	0.01	36
15 December 1966	0.01	32

TABLE IV. ACTIVITIES OF THE SAMPLES OF BLOOD OF GUINEA-PIG 2

Date	Volume of blood (ml)	Average activity (in counts/min)
9 December 1966	0.01	132
10 December 1966	0.01	80
12 December 1966	0.01	96
13 December 1966	0.01	128
14 December 1966	0.01	38
15 December 1966	0.01	38
17 December 1966	0.01	32

geometry in relation to a Geiger-Müller counter placed on the opening of the glass tube.

3.2.2. Results of the labelling of adults

The results obtained with guinea pig 2 are shown in Table V. As was expected, the activity of the flies was little but easily detectable.

In a first test of glossina labelling it was noticed that the glossina continued to be radioactive after 13 days, as was readily detected with a Geiger-Müller counter. We therefore consider this technique adequate for labelling glossina as a contribution to the study of their behaviour in nature.

However, this method must be improved so that a higher radioactivity count may be obtained for each fly. Attempts are therefore being made to label the glossina by contact by means of a piece of filter paper soaked in a mixture of ^{32}P and risela oil. The glossina are put in contact with the radioisotope by means of a tubular cage, the model of which was recommended

for insecticide research by the WHO and is used to apply the chemosterilants by contact in a similar way.

3.2.3. Attempt at labelling spermatozoa

Assuming that the food absorbed together with the ingested blood must reach every organ of the insect, including the testes, we hope to mark the spermatozoa. For this purpose we fed glossina males on the labelled guinea-pigs, forcing the flies to feed daily to obtain the largest possible concentration of ^{32}P in the haemocoel. The testes were isolated and placed on glass plates for the radioactivity counting. No detectable activity was reported and therefore the autoradiographic observations we had in mind were not performed.

As ^{51}Cr labels erythrocytes, a new test was devised in which ^{51}Cr was substituted for ^{32}P to label the guinea-pig on which the glossina would feed. However, as this test is still in its initial phase no results are yet available.

3.3. Glossina flora

Like all other creatures, glossina have their own flora, more or less virulent according to circumstances. Moreover, various microbial agents have already been reported on glossina, some of which, such as Bacterium mathisi [8, 9], have proved to be pathogenic.

TABLE V. ACTIVITIES OF A MALE G. morsitans FED ON GUINEA-PIG 2

Date	Blood meals	Average activity (in counts/min)
14 December 1966	Before the meal	-
	After the meal	65
15 December 1966	Before the meal	62
	After the meal	143
16 December 1966	Before the meal	74
	After the meal	138
17 December 1966	Before the meal	Not measured
	After the meal	127
20 December 1966	Before the meal	163
	After the meal	186
21 December 1966	Before the meal	167
	After the meal	223
22 December 1966	Before the meal	155
	After the meal	195
26 December 1966	Before the meal	108
	After the meal	143

Note: The fly died on 27 December 1966.

To ascertain the nature of our colony's flora and with the possibility of discovering some microbial species able to interfere with the glossina life cycle and thus capable of being used for biological control, we initiated the study of the glossina flora with the co-operation of the Câmara Pestana Bacteriological Institute (Professor Cândido de Oliveira and Dr. Gustavo Nobre). By this work, we hope to contribute to the knowledge of the real role of the bacteroids that have been found in the midgut of these insects as well as of the influence of bacteria in some aspects of anormal evolution such as premature death and dystocia.

This work is still in its initial phase since cultures so far have been made from six glossina specimens only. For the cultures we have collected not only the flies' intestinal contents but also their tegument, cultures of both being made in solid and fluid media, with and without blood, incubated aerobically and anaerobically at different temperatures and also in media for fungi. The microbial strains that have been isolated are still under observation.

4. CONCLUSIONS AND LINES OF WORK

We can state that the problem of the breeding of G. morsitans morsitans in the laboratory has been successfully solved. We have a progressive colony of this species and will place it gladly at the disposal of all concerned. We are now facing a large number of investigations, concerning both a more comprehensive knowledge of the insect's physiology and biology as well as of the means of biological control, and the study of the details pertaining to its role as a vector.

As far as the practical application of our colony is concerned, we can report the studies already performed on some aspects of the biology of the species, on the sexual aggressiveness of the males bred in captivity in comparison with wild males, and on the radioisotope labelling that is easily carried out to study the insect's dispersion and length of life in nature, as well as possibly a means of studying cross-breeding between species or sub-species.

We hope to improve our capabilities to a much greater extent and are counting on having the necessary resources soon, such as an increase and improvement in our present facilities and an adequate staff for the work we intend to perform.

Among the studies we expect to carry out, two have top priority: the maintenance of polymorphic trypanosomes in the laboratory via the glossinic cycle, and studies concerning the factors that determine glossina feeding and reproduction, in particular lighting conditions, temperature and humidity.

With the former objective we hope to contribute to the knowledge of the antigenicity, immunology and sensitivity to drugs of the trypanosomes in their natural cycle, and possibly to the preparation of a vaccine; with the latter studies we intend to determine what are the qualitative and quantitative nutritional demands of the glossina and which elements intervene in the metabolism of their basic principles, especially those concerning their hydric metabolism.

The continuation of the studies concerning the application of chemosterilant procedures and the resulting biological changes, as well as

studies concerning the insect's sensitivity to insecticides, and other subjects, will be also considered.

5. SUMMARY

Bruce's initial and valuable work in 1895 was the first attempt at the laboratory breeding of the tsetse fly. However, the problem was solved only after 1959 with the successful breeding of G. morsitans in Lisbon.

From 1959 to the present, the initial colony has been constantly increasing, as the accident with an insecticide caused only a temporary decrease of the insect population in 1965.

However, whatever the explanation of our success (genetic causes or the technique employed), we believe we may safely conclude that our method guarantees the breeding of G. morsitans. To the constantly successful progress of the Mozambiquan colony of 1959, we may now add the equally good results obtained with a new colony, initiated with Rhodesian specimens, that has also been growing progressively since its initiation.

Another G. sub-morsitans colony started in May 1966 has also been satisfactorily developing and is now in the 9th generation.

Since the breeding of G. morsitans is now assured, it has become possible to study some of the insect's biological and physiological characteristics. A study of the possibility of employing sterilized males, bred in captivity, in the biological control of tsetses has also been initiated[3].

We are also attempting to label males of G. morsitans with radioactive phosphorus to achieve spermatozoa marking for the study of the possible occurrence of cross-breeding among different species and subspecies, through the radioactivity present in the spermathecas.

We have also initiated a study of the bacterial and mycological flora of G. morsitans in adequate media to ascertain its influence on normal glossina growth, as well as on the pathological situations sometimes observed.

In view of the successful evolution of our colony, as well as its already large volume (at present we have about 2400 specimens), we also intend to study the factors affecting glossina infection by polymorphic trypanosomes, both orally and rectally, as well as a study of the antigenicity of those cyclically transmitted in the laboratory.

Our immediate lines of research also include some other physiological aspects of the glossina, mainly concerning their nutritional needs.

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[3] DAME, D.A., "The present situation and future prospects for control of tsetse by the sterile-male technique", these Proceedings; DAME, D.A., "Chemosterilization, radiosterilization, rearing and ecological studies of Glossina", these Proceedings.

REFERENCES

[1] NASH, T.A.M., JORDAN, A.M., BOYLE, J.A., A promising method for rearing Glossina austeni (Newst.) on a small scale, based on the use of rabbit's ears for feeding, Trans. R. Soc. trop. Med. Hyg. 60 2 (1966) 183.

[2] ITARD, J., MAILLOT, L., Notes sur un élevage de Glossines (Diptera-Muscidae) entrepris, à partir de pupes expédiées d'Afrique, à Maison-Alfort (France), Rev. Elev. Méd. vét. Pays trop. 19 1 (1966) 29.

[3] FRAGA DE AZEVEDO, J., PINHÃO, R., The maintenance in the laboratory of a colony of Glossina morsitans (Diptera) since 1959, Proc. XIIth Int. Congr. Ent., London (1965) 780.

[4] FRAGA DE AZEVEDO, J., PINHÃO, R., The maintenance of a laboratory colony of Glossina morsitans since 1959, Bull. Wld Hlth Org. 31 (1964) 835.

[5] WILLET, K.C., Personal communication (1965).

[6] MacDONALD, W.A., The laboratory rearing of Glossina morsitans submorsitans Newst. in Northern Nigeria, Int. scient. Com. Tryp. Res., 8th meeting, Jos, CCTA, publication 62 (1960) 247.

[7] JENKINS, D.W., "Radioisotopes in entomological studies of endemic and tropical diseases, Radioisotopes in Tropical Medicine (Proc. Symp. Bangkok, 1960), IAEA, Vienna (1962) 235.

[8] ROUBAUD, E., TREILLARD, M., Un coccobacille pathogène pour les mouches tsétsés, C.r. hebd. Séanc. Acad. Sci. Paris, 201 (1935) 304.

[9] ROUBAUD, E., TREILLARD, M., Infection expérimentale de Glossina palpalis par un coccobacille pathogène pour les muscides, Bull. Soc. Path. exot. 29 (1936) 145.

EFFECT OF GAMMA RADIATION ON THE FERTILITY OF TORSALO, Dermatobia hominis (DIPTERA: CUTEREBRIDAE)

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Abstract

EFFECT OF GAMMA RADIATION ON THE FERTILITY OF TORSALO, Dermatobia hominis (DIPTERA: CUTEREBRIDAE). Research on the possible application of the sterile-male technique for controlling the torsalo required observations on the effect of gamma radiation on its fertility. Torsalo pupae, 20, 25 and 30 days-old, were exposed to gamma radiation from a ^{60}Co source. Three dosages, of 5000, 7500, and 10 000 R, were used. These exploratory investigations showed that the most satisfactory time for the treatment was 25 days, representing 5/7 of the pupal development at 25°C. All dosages used drastically reduced the fertility of the flies, but the 7500 R dose, which completely inhibits the development of ova in the females and reduces the fecundity of the males to 0.8%, is the appropriate dose for practical purposes. The sperm of the treated males retained its motility. The appearance and behaviour of the treated flies was apparently normal.

The success of the sterile-male method of eradicating the screw-worm, Callitroga hominivorax (Coquerel), from Curaçao and the south-eastern United States of America [1], has suggested the possibility of using sexually sterile insects for control or eradication of other species [2, 3].

Several workers have pointed out that this method might be a feasible way to control Dermatobia hominis (L., Jr.), the torsalo, the larvae of which are obligatory parasites in warm-blooded animals, including man, and which in the tropical Latin American countries represent a serious handicap to the cattle industry. For reviews on the biology of the torsalo, see Refs [4-6]. There is a discussion on the possibilities of a sterile-male programme in Ref. [7].

The data presented here are the results of the preliminary tests conducted to determine the effects of gamma radiation on the fertility of the torsalo.

MATERIALS AND METHODS

The test insects originated from the laboratory colony and from cattle naturally infested in the field. In both instances fully grown larvae that voluntarily left their host were collected, and the larvae and pupae were handled in essentially the same manner as described in Ref. [7].

Batches of pupae of different ages were subjected to doses of 5000, 7500 and 10 000 R of gamma radiation from a ^{60}Co source, emitting 56 R/min. We are aware that the dose-rate of 56 R/min, which was the limit with the available equipment, was low, and that the effect may be increased by increasing the intensity [8].

The emerging flies used in mating experiments were separated according to sex every half hour to ensure that they were not previously mated. The mating and "egging" procedures, described in Ref. [7], will be summarized here. When flies were 1-d old, 4 to 6 of each sex were confined together in 30 × 30 × 30-cm breeding cages. After 6 h the males were removed and the next day the cages were provided with a number of Musca domestica L. on which the eggs were laid. The female torsalo were examined for the presence of sperm in the spermathecae when they appeared moribund. The eggs were incubated at 28°C and 100% r.h., at which temperature they started hatching after 5 d. Hatching was induced by placing the eggs in the palm of the hand and blowing on them.

RESULTS AND DISCUSSION

Because there were few pupae, tests were initiated with a dose of 5000 R, which in related species was adequate to induce sterility. Pupae that had completed 4/7, 5/7 and 6/7 of the pupal period, and were therefore representative of different stages of pupal development, were irradiated.

The length of the pupal period was not affected by irradiation (Table I). A dose of 5000 R apparently had no effect on the emergence regardless of the age of the pupae when treated. When the dose was raised to 7500 R few flies emerged from treated 20-day-old pupae, whereas the emergence of flies from the treated 25- and 30-day-old pupae was normal. Emergence of flies treated as 25-day-old pupae was normal at 10 000 R, and it was concluded that pupae having completed 5/7 or more of their pupal development emerge normally when subjected to the dosages described, whereas pupae having completed only 4/7 of their development suffer mortality attributable to irradiation at doses higher than 5000 R.

The flies that emerged from irradiated pupae were normal in appearance and behaved like non-irradiated flies. The percentages of females inseminated in these crosses (N = not radiated; R = radiated), ♀ N × ♂ N, ♀ N × ♂ R, and ♀ R × ♂ N, were respectively 83.3, 80.3, and 81.5%, suggesting that the treated males mate as readily as the untreated. Longevity of the adults, which under laboratory conditions is short (3-4 d), was apparently not affected by the treatment.

The results of the mating tests indicated that exposure to gamma radiation during the pupal period drastically affected the fertility of male and female torsalo flies. An age of 25 d (5/7 of the pupal development) appeared to be the best age for the treatment. In the flies treated as 30-day-old pupae the fertility was less reduced and, as mentioned above, in irradiated 20-day-old pupae somatic damage was great.

The following refers to flies treated as 25-day-old pupae. Treated with 5000 R the females oviposited about 6% compared with the controls. The egg masses were small, not arranged normally on the vectors and only 6% hatched. At 7500 R the females were sterilized, the development

TABLE I. EFFECT OF DIFFERENT DOSES OF GAMMA RADIATION ON FLY EMERGENCE AND LENGTH OF PUPAL PERIOD WHEN PUPAE WERE TREATED AT DIFFERENT AGES

Age of pupae when treated (d)[a]	Dosage (R)	Number of pupae in test	Number of flies emerged ♀	Number of flies emerged ♂	Emergence (%)	Average pupal period (d)[b] ♀	Average pupal period (d)[b] ♂
30	5000	80	26	28	67.5	38	36
	untreated	67	25	27	77.6	37	36
25	5000	60	22	21	71.7	36	35
	untreated	50	13	19	64.0	36	35
20	5000	70	15	29	62.9	36	35
	untreated	65	21	31	80.0	36	35
30	7500	72	24	17	56.9	36	35
	untreated	40	15	11	65.0	36	35
25	7500	50	20	19	78.0	36	35
	untreated	41	15	21	87.8	36	35
20	7500	55	3	2	9.1	37	36
	untreated	45	19	11	66.7	36	35
25	10 000	65	30	19	75.4	36	35
	untreated	68	29	27	82.4	36	35

[a] From the day larvae leave the host until treated.

[b] From the day larvae leave the host until adults emerged from pupae.

of mature ova being completely inhibited. Females irradiated in the pupal stage always had smaller ovaries than the controls. The ovaries normally are fully developed at the time the flies hatch. The damage to the gonads was accentuated as the dosage was increased from 5000 to 10 000 R. The average volume of one of the two ovaries measured in 10 females treated with 10 000 R as 25-day-old pupae was about 5 mm^3 against about 16 mm^3 in the controls.

Untreated females mated with males exposed to from 5000 to 10 000 R, and virgin females, deposited the normal number of eggs. When normal females were mated to males treated with 5000 R, hatchability was 1.3% or 3.6 eggs hatched per female inseminated. When normal females were mated to males treated with 7500 R, hatchability was 0.8%, and by increasing the dose to 10 000 R, 0.4% of the eggs fertilized by these males hatched.

The size of the testes was apparently not affected by the treatment and motile sperm were found at all the dosages. The spermathecae of females inseminated by treated males contained sperm in a number apparently equal to that of the controls, the sterility in the males thus being due to the gametes containing dominant lethal changes, but retaining motility and vigour.

CONCLUSIONS

Results of tests in which torsalo pupae of different ages were exposed to irradiation from a ^{60}Co source showed that no practical difficulties exist regarding sterilizing this species by means of ionizing radiation. The tests suggest that a dose of 7500 R applied to pupae that have completed 5/7 of their development was a sterilizing dose.

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REFERENCES

[1] BUSHLAND, R. C., "Screw-worm research and eradication", in Advances in Veterinary Science 6, Academic Press, New York (1960) 1.

[2] KNIPLING, E. F., Possibilities of insect control or eradication through the use of sexually sterile males, J. econ. Ent. 48 (1955) 459.

[3] The potential role of the sterility method for insect population control with special reference to combining this method with conventional methods, Rep. ARS-33-98, US Department of Agriculture, Washington, D. C. (1964).

[4] NEEL, W. W., URBINA, O., VIALE, E., de ALBA, J., Ciclo Biologico del tórsalo (Dermatobia hominis L. Jr.) en Turrialba, Costa Rica, Turrialba 5 (1955) 91.

[5] KOONE, H. D., BANEGAS, A. D., Biology and control of Dermatobia hominis in Honduras (Diptera: Cuterebridae), J. Kans. ent. Soc. 32 (1959) 100.

[6] ANDERSEN, E. H., Control of Dermatobia hominis in America, Vet. Rec. 74 (1962) 284.

[7] BANEGAS, A. D., MOURIER, H., GRAHAM, O. H., Laboratory colonization of Dermatobia hominis (Diptera: Cuterebridae) (unpublished).

[8] RHODE, R. H., LOPEZ, F., EGUISA, F., TELICH, J., Effect of gamma radiation on the reproductive potential of the Mexican fruit fly, J. econ. Ent. 54 (1961) 202.

CHEMOSTERILIZATION, REARING AND ECOLOGICAL STUDIES OF Glossina

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Abstract

CHEMOSTERILIZATION, REARING AND ECOLOGICAL STUDIES OF Glossina. Tsetse flies were successfully sterilized by various chemosterilants, and their competitiveness was found to be normal. Two colonies are being maintained in the laboratory. Ecological studies were conducted for one year on a natural population. A release of sterile males is expected shortly.

Injections of 1 μl of aqueous solutions of sterilants into the pronotum of 0 to 24-hour-old G. morsitans males produced 100% sterility with 1 μg of tepa or 5 μg of metepa. Apholate produced a high degree of sterility with 5 μg, but was toxic at higher levels. Hempa failed to sterilize at 20 μg, and was toxic at higher levels.

Contact exposure to tepa or metepa permanently sterilized adult G. morsitans males of various ages with 30 to 240-min exposures to deposits of 10 mg/ft^2 on glass. These sterile males were fully competitive with untreated males in the laboratory. Fifteen-minute exposure to tepa allowed a slight recovery of fertility after a few matings. Longevity was normal with exposures of 60 min or less, but 240-min exposures reduced male longevity by 17 and 33% with metepa and tepa respectively. Hempa failed to sterilize by contact exposure and apholate gave inconsistent results, even though deposits of both sterilants were increased to 100 mg/ft^2.

Exposure of G. morsitans males of various ages to wind tunnel applications of 0.25 ml of 5% tepa in methanol produced permanent sterility. The same exposure to metepa permanently sterilized two-day-old males, but some recovery of fertility occurred with freshly emerged and nine-day-old males. Longevity was not seriously affected with two-day-old males and competitiveness was normal with both tepa and metepa treatments. Neither hempa nor apholate was tested in this manner.

Dipping G. morsitans pupae in 5% solutions of tepa in 50% aqueous methanol resulted in permanent male sterility in flies emerging during the first two post-treatment weeks, but subsequent emergees were not completely sterile. Longevity and competitiveness were unaffected. Similar exposure to metapa produced complete male sterility, but apholate and hempa were ineffective.

G. morsitans males sterilized with tepa by 60-min contact or in the wind tunnel were competitive under field cage conditions, and dispersed normally in field release trials.

In multiple mating experiments G. morsitans females copulated with more than one male. Males mated several times, but after the fourth or fifth occasion the mating tended to be infertile.

Injection of sterilants into G. pallidipes males resulted in complete sterility with 1 μg tepa and 5 μg metepa. Permanent male sterility of G. pallidipes was achieved without affecting longevity or competitiveness by a 30-min exposure to metepa deposits of 10 mg/ft^2 or by spraying with 0.25 ml of 5% tepa in methanol in the wind tunnel.

Laboratory studies demonstrated that chemosterilized G. morsitans males are capable of transmitting Trypanosoma congolense. When the flies were treated before becoming infected, 50% of the host animals became infected; when the flies were treated after becoming infected, 17% of the host animals became infected, and 100% of the host animals became infected in the untreated controls.

Two self-sustaining colonies of G. morsitans are being maintained in the laboratory. One colony is more than two years old and the second colony is one year old. Selection for pupal weight in the first generation of the second colony seems to have been helpful.

An isolated field population of G. morsitans has been under surveillance for 18 months. Population densities are estimated at 3700-5300 males/mile^2 depending on the season. Male birth rates decreased from 560/mile^2 per day in November to 110 in August, whereas male survival over a four-day period increased from 69% in November to 96% in August. Because of the offsetting birth and mortality trends, the population was relatively stable for most of the year. This population will be subjected to the release of sterile males during 1967.

THE PRESENT SITUATION AND FUTURE PROSPECTS FOR CONTROL OF TSETSE BY STERILE-MALE TECHNIQUES

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Abstract

THE PRESENT SITUATION AND FUTURE PROSPECTS FOR CONTROL OF TSETSE BY STERILE-MALE TECHNIQUES. Techniques are available for sterilizing G. morsitans to be used in experiments with sterile-male releases. A pilot test is in preparation. It is likely that research making use of similar techniques (with slight alterations in exposure rates) will provide suitable sterile males of other tsetse fly species. Extensive control programmes must await the development of larger self-sustaining colonies of the flies.

The theoretical potential of the sterile-male release technique for control of tsetse flies (Glossina spp.) was outlined by Simpson [1] and Knipling [2, 3].

In describing their theories, these authors made many assumptions with regard to the reproductive behaviour and population dynamics of the flies. Although these assumptions were based on a reasonably sound knowledge of tsetse fly behaviour, the applicability of the sterile-male technique to Glossina can be demonstrated only by actual trials under field conditions. Substantial progress has been made in this direction. The most critical need is development of self-sustaining colonies of the flies to provide males in sufficient quantities for large-scale releases. The use of sterile-male tsetse flies might be contemplated for three types of situations: (1) In controlling low-level, established populations; (2) in conjunction with insecticides, bush clearing and other control methods that will first reduce the natural population to levels manageable by the release of sterile males; (3) in preventing the spread of tsetse flies into new areas. It is too early in the research programme to outline the needs for successful application of the sterile insect technique for eradication of the tsetse fly. Much further research needs to be conducted and several problems need to be resolved. However, it might be worth while to consider the general magnitude of sterile insect release programmes that might be involved. Some of the progress in research to date will be discussed, and also some of the possibilities and probable requirements for the practical application of this method of tsetse fly control.

Early work by Potts [4] demonstrated that a high degree of sterility could be obtained by exposing pupae of G. morsitans to gamma-irradiation from ^{60}Co. In subsequent trials Dean confirmed Potts' work [5] and obtained permanent sterility of 93-100% in males of G. morsitans by exposing pupae to 7500-15 000 rad of gamma irradiation. These males were

competitive when tested with untreated males in the laboratory and in outdoor cages. Mortality of pupae due to radiation damage was substantially reduced by improved timing of the exposure in relation to pupal age, and sterilization of adult males was successful (G. J. W. Dean, personal communication).

The effect of chemosterilants on tsetse flies was also investigated. Chadwick [6] reported that apholate and metepa failed to sterilize completely G. morsitans even at dosages that produced excessive mortality. Further work in our laboratory showed that both metepa and tepa were effective and versatile sterilants, but that apholate and hempa were ineffective. Laboratory trials demonstrated that contact or spray (wind-tunnel) exposures to either tepa or metepa produced permanent sterility without reducing the male life span or sexual competitiveness. Both chemicals were also effective when used as pupal dips. Males treated with tepa by contact or with a spray were tested in field cages and also released in the field in their natural habitat. In both circumstances the sterile males performed as well as those untreated.

After these promising results in sterilizing the flies, we are now in a position to conduct a pilot field operation with sterile males of G. morsitans within an isolated population. For more than a year detailed observations of this population provided information about seasonal fluctuations in population density, birth and survival rates, and reproductive status of the females in the population. We expect to conduct a release programme with sterile males obtained from pupae collected in the natural habitat.

However, the natural habitat cannot be considered a suitable source of tsetse flies for large-scale use of the sterile-male technique. It is generally recognized that a successful method of rearing tsetse flies is necessary before this technique can be used to eradicate the insects. In the laboratory, successful colonization of G. morsitans was achieved by Azevedo and Pinhão [7] and of G. palpalis by Geigy [8]. The invaluable techniques and knowledge acquired through these and other efforts were adequately reviewed by Nash [9]. Since 1963 Nash and other workers have made further progress with laboratory rearing of G. austeni and G. morsitans. The poor adaptability of flies to laboratory conditions prompted many workers to maintain them in individual containers for colonization. It must be readily apparent that colonies of individually held females are not suitable for producing large numbers of flies, although they may be quite adequate in supporting research needs. Colonies with large numbers of reproducing females seem to offer the best potential for mass production of tsetse flies. McDonald [10], who held groups of females in small cages, achieved excellent production of pupae, although the colony was not self-sustaining. Currently, the Agricultural Council of Central Africa is exploring the possibility of mass rearing G. morsitans under field conditions in both large and small cages with a variety of host animals. In addition, attempts are being made to build up the natural density of tsetse flies by increasing the population of the host animals, with the hope that we can harvest a crop of pupae. Additional rearing experiments are being conducted in climate-controlled environmental chambers.

An inherent danger in colonizing insects is the potential loss of behavioural characteristics essential for survival in the field. It is impossible to predict the behaviour of colonized flies in the field. However, with the

mosquito Anopheles quadrimaculatus, the severe selective pressures of colonization were felt to be the cause of poor performance by sterilized males when they were released in the natural habitat [11]. One cannot categorically state that species not easily colonized are prone to severe selection during the process of colonization, but it seems likely that such tendencies favour accumulation of behavioural characteristics that produce successful perpetuation in confinement. In this connection, we are now conducting a series of tests to determine the behavioural characteristics of Azevedo's colony of G. morsitans. This colony appears to have survived substantial selective conditions during several years and may provide evidence of the effect of colonization on the behaviour of G. morsitans. Even though we need to study carefully any behavioural changes in laboratory-adapted strains, such adapted strains may still allow us to achieve our purpose when they are released, just as laboratory-adapted screw-worm flies and tropical fruit flies are being employed successfully in sterile insect release programmes.

Following this general outline of the current status of investigations of the sterile-male technique with regard to tsetse flies, perhaps it would now be useful to consider the numbers of sterile males needed to conduct control operations. Knipling [3] postulated that quarterly release rates of 300, 150, 75 and 37 sterile males per square mile per month might eradicate low-density (200 per square mile) populations. He also suggested that sterile-male releases might be employed after applying non-residual insecticides to reduce populations of a higher natural density (up to 1000 per square mile). By using these estimates as a basis for calculation, we can estimate that the projected release of 1700 males per square mile over an area of 1000 square miles would require 1 700 000 sterile males to attain eradication in one year. By suitable programming, sterile males produced in mass rearing systems could be most efficiently utilized by initiating control operations in a new block each quarter (Table I). Owing

TABLE I. ESTIMATED NUMBER OF STERILE TSETSE FLY MALES NEEDED TO CONDUCT AN ERADICATION PROGRAMME COVERING A NEW 1000-SQUARE-MILE AREA EACH QUARTER[a]

Months	Number of males released per month in 1000-square-mile block					Total number released per month
	Block					
	A	B	C	D	E	
1-3	300 000	-	-	-	-	300 000
4-6	150 000	300 000	-	-	-	450 000
7-9	75 000	150 000	300 000	-	-	525 000
10-12	37 000	75 000	150 000	300 000	-	562 000
13-15[b]	-	37 000	75 000	150 000	300 000	562 000

[a] These estimates can be converted directly into estimates for larger or smaller areas.
[b] Programme following similar pattern continues indefinitely.

to the vast areas of infestation and the naturally low reproductive capacity of the tsetse fly colonies that would be used to provide sterile males, it is unlikely that large infestations of tsetse flies could be eradicated in a single year. However, with the system outlined above, it would be possible to decrease a tsetse infestation in gradual steps. Under ideal conditions this type of approach could eliminate the need for follow-up releases since progression along the tsetse fly front would prevent successful re-invasion. It is therefore evident that the total area which could be brought under control each year by the release of sterile males would be limited by the number of male flies that could be reared. The number, in turn, would be dependent on our current knowledge and progress in colonizing the flies. The use of sterile-male releases will require very efficient techniques of mass production.

An alternative method in using the sterility principle to control Glossina spp. would be to lure the natural population to the chemicals for self-sterilization. By sterilizing the natural population directly, the need for large colonies would be obviated. Theoretically, the flexibility provided by using a chemosterilant opens the way for several such alternative methods. It must be remembered, however, that the effective chemicals now available may be highly mutagenic to both plants and animals. The use of any attractant-chemosterilant combination would have to be selective enough to minimize or prevent exposure of organisms other than Glossina spp. To date there are no chemical attractants that offer immediate promise for Glossina. Physical factors such as light, sound, etc., either alone or in combination with movement of host animals, might provide some degree of attraction. In any event, the attractant-sterilant combination would probably be most efficient at high population densities and less efficient at low population levels [3]. It is likely that the release of sterile males would be desirable to attain maximum efficiency during the final stages of an attractant-sterilant programme. Thus, self-sustaining colonies of tsetse flies reared to furnish males for sterilization might still be highly advantageous in such eradication efforts. We might expect that under these conditions the numbers needed to supplement the major technique would be very small per unit area. Therefore, relatively large areas of infestation could be flooded with males from supporting colonies comparable in size to those needed to conduct a straightforward sterile-male release programme in smaller areas.

The suitability of the sterile-male technique will undoubtedly vary in relation to the species to be controlled. In this respect, several factors, including population density, behaviour and distribution, will have an important bearing. In addition, each species considered must be subjected to laboratory evaluation of sterility before field trials. We found, for example, that G. pallidipes can be sterilized by the same chemicals as G. morsitans. However, the behaviour and life span of G. pallidipes were more readily impaired by the treatment than the behaviour and life span of G. morsitans. Such variations in the tolerance of species may prove to be a very important factor.

REFERENCES

[1] SIMPSON, H.R., The effect of sterilized males on a natural tsetse fly population, Biometrics 14 (1958) 159.

[2] KNIPLING, E. P., Potential role of the sterility principle for tsetse fly eradication, WHO unpublished mimeographed document (WHO/Vector Control/27, WHO/EBL/9, 1963).
[3] KNIPLING, E. F., "The sterility principle of insect population control", 12th International Congress of Entomology, London.
[4] POTTS, W. H., Sterilization of tsetse flies (Glossina) by gamma irradiation, Ann. trop. Med. Parasit. 52 (1958) 484.
[5] DAME, D. A., DEAN, G. J. W., FORD, J., "Investigations on the sterile male technique with Glossina morsitans", Proc. 10th Meeting of the International Scientific Committee for Trypanosomiasis Research, Kampala, Uganda, 93.
[6] CHADWICK, P. R., Effect of two chemosterilants on Glossina morsitans, Nature, Lond. 204 (1964) 299.
[7] AZEVEDO, J. F., de PINHÃO, R. C., The maintenance of a laboratory colony of Glossina morsitans since 1959, Bull. Wld Hlth Org. 31 (1964) 835.
[8] GEIGY, R., Elevage de Glossina palpalis, Acta Trop. 5 (1948) 201.
[9] NASH, T. A. M., Progress and problems in the establishment and maintenance of laboratory colonies of tsetse flies, Bull. Wld Hlth Org. 28 (1963) 831.
[10] McDONALD, W. A., "The laboratory rearing of G. morsitans submorsitans in Northern Nigeria, Proc. VIIIth Meeting International Scientific Committee for Trypanosomiasis Research, Jos, Nigeria (1960) 247.
[11] DAME, D. A. et al., Field behavior of sexually sterile Anopheles quadrimaculatus males, Mosquito News 24 (1964) 6.

STERILIZATION WITH GAMMA-RAYS AND FIELD INVESTIGATIONS INTO THE BREEDING OF Glossina morsitans

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Abstract

STERILIZATION WITH GAMMA-RAYS AND FIELD INVESTIGATIONS INTO THE BREEDING OF Glossina morsitans. Gamma-radiation between 8000 and 15 000 rad to pupae of Glossina morsitans of unknown age collected in the field reduced reproduction in male flies emerging within a week of treatment when mated with untreated females by more than 95%. Male flies emerging from pupae during the second and third post-treatment weeks were completely sterilized with dosages of 9000 and 4000 rad respectively. Female flies were completely sterilized by 1000 rad. The survival of irradiated pupae and subsequent adult flies was reduced both by increasing dosage and by decreasing pupae age at the time of treatment, male flies being more sensitive than females to gamma-rays.

Adult male flies irradiated with 8000 to 16 000 rad soon after emergence also showed about a 95% reduction in fertility, but they lived as long as the untreated flies. Sterility was permanent during 45-day test periods. The sperm from irradiated males were motile and apparently behaved normally, and insemination was good. Laboratory and field cage trials indicated that irradiated males could compete successfully with untreated males and reduce reproduction from untreated female flies.

Present difficulties in breeding the number of tsetse flies necessary for a practical sterile-male release programme are being investigated in the Zambezi Valley, but it is too early to indicate success. The possibility of concentrating the breeding of a natural fly population is being explored by localizing cattle as a food source within the bush. Cement pipes are being used to simulate natural laviposition sites in an attempt to concentrate breeding. Eight-feet-high, quarter-acre wire-mesh cages enclosing the lower bush canopy, a 30-feet-high cage round an evergreen tree, and a variety of smaller wire-mesh and cement brick cages exposed to variable field, and relatively constant, climates have been erected to study fly survival and reproduction. Oxen are usually employed as the host in these cage trials but a separate study will compare survival and reproduction obtained from a variety of indigenous and domestic animals. These sterilization and breeding projects are supported by more than one million pupae collected by and knowledge gained from Dr. Phelps' associated study of natural breeding sites used during the year.

In 1964, the Agricultural Research Council of Central Africa, in association with the United States Department of Agriculture and the International Atomic Energy Agency, began to investigate the possibility of using the sterile-male release principle as a practical means of controlling the most important tsetse fly species, Glossina morsitans. Dr. Dame has described the progress made using chemicals as the sterilizing agent and the preliminary field work necessary for an eradication trial on an island in Lake Kariba[1]. This contribution concerns research on radiation-sterilization and the field problems in breeding tsetse.

Investigations into the effects of gamma-radiation on tsetse were begun in 1964 under a co-operative agreement with the International Atomic Energy Agency who supplied an Eldorado Cobalt-60 Teletherapy Unit to the Central Hospital in Salisbury. Supplies of pupae were obtained each week from

[1] DAME, D.A., "Chemosterilization, rearing and ecological studies of Glossina", these Proceedings.

Dr. Phelps' study into natural breeding sites in the Zambezi Valley. These pupae were of all ages. The effects of gamma-radiation were tested both on these pupae of unknown ages and on the adult flies emerging in the laboratory.

The pupae were held in fine cotton mesh bags during treatment, and the adult flies were enclosed in small wooden-framed boxes covered with cotton mesh. The practical dose-rates that could be obtained from this radiation unit were relatively low, varying between 54 and 122 rad/min. Total doses of gamma-radiation between 1000 and 16 000 rad were investigated. The laboratory procedures used to compare sterility, longevity and competitiveness of treated and untreated flies were standardized with those used in the chemosterilization studies.

The results obtained from the pupal treatments showed that the level of male sterility increased with dosage but there were some complications. It was possible to obtain only about 95 - 97% sterility with males subjected to 8000 to 15 000 rad within a week of eclosion. Males emerging from pupae during the second and third weeks after treatment were completely sterilized with 9000 and 4000 rad respectively. However, these levels of gamma-radiation were found to increase mortality both among the pupae and among the adults emerging from the treated pupae. Total mortality of these mixed-aged pupae due to radiation increased from 23% at 2000 rad to 64% at 15 000 rad. Pupae of known ages from a laboratory colony were used in some tests and these showed that 8000 rad did not noticeably affect pupal survival when they were between 17 and 29 days old at the time of treatment. However, mortality rapidly increased when progressively younger pupae were treated, and none survived when irradiation occurred during the first ten days of pupal life. All the tests, both with known- and unknown-aged pupae, showed that the male pupae were more sensitive to gamma-rays than the females. The longevity of the adult fly emerging from treated pupae also decreased with a decrease in pupal age at the time of irradiation, and with an increase in the total radiation dose.

The timing of pupal treatments is therefore important, and it would be essential to have pupae of known age from a successful breeding system. However, it seems unlikely that completely sterilized male flies will be obtained without an excessive wastage of pupae and a reduced adult life span. Fractionated dosages were tested in an attempt to reduce this mortality, but the improved life span compared with the flies given a continuous but similar total dosage is still shorter than that obtained with the untreated flies. In contrast, the female flies are completely sterilized by 1000 rad, and live longer than the males given a similar dosage.

The situation is improved by irradiating adult males within a week of eclosion. The 95% sterility obtained with dosages up to 16000 rad was comparable with the results achieved by treating pupae up to a week before eclosion. However, the treated adult males appeared to survive as well or better than untreated flies for the first seven weeks. In all the tests on reproduction it was found that the treated and mated males lived longer than the virgin males.

Table I summarizes the results obtained when 12 000 rad were given to the males a week before, and within a week after, eclosion. The survival of the adult males, and the percentage reduction in the number of progeny produced when mated with untreated females, showed that treating the adult male was more promising than irradiating the pupae, and has two

TABLE I. MEAN SURVIVAL OF VIRGIN MALE Glossina morsitans AND MEAN NUMBER OF PROGENY OBTAINED AFTER EXPOSURE TO 12 000 RAD (a) DURING THE LAST WEEK OF PUPAL LIFE, AND (b) AFTER ECLOSION

	Treatment	Survival during week (%)								Mean No. of pupae	Mean No. of progeny	Reduction in reproduction (adults) (%)
		1	2	3	4	5	6	7	8			
(a)	Irradiated pupae	72	48	28	16	11	4	2	0	0.6	0.5	97.5
	Untreated pupae	81	67	54	43	30	25	22	14	23.0	20.0	-
(b)	Irradiated adults	83	67	50	45	28	31	21	6	1.7	1.0	95.0
	Untreated adults	90	68	55	30	23	18	10	10	20.5	20.0	-

added advantages. Firstly, there is no wastage of pupae due to mortality from gamma-radiation. Secondly, there is no wastage of females, as these can be returned to a breeding scheme; in the pupal stage the females cannot be separated from the males.

Laboratory tests in 8 in. × 8 in. × 11 in. cages showed that irradiated males competed with untreated males for untreated females at a ratio of 4:1:1, and reduced reproduction by about the expected 80%. Field tests with a similar ratio of treated and untreated flies were done in an 8 ft × 12 ft × 24 ft cage. The males were allowed to compete for the females for two days and the females were then recaptured and held in the laboratory for evaluation of reproduction. These field tests gave results similar to those obtained in the laboratory, and closely resembled the results obtained by Dr. Dame with males sterilized with chemicals.

FIELD INVESTIGATION INTO BREEDING OF TSETSE

A practical sterile-male release programme against natural tsetse populations requires an efficient system for supplying viable flies for treatment. It is impractical to rely on catching flies from the bush even though the males are attracted to tethered oxen or a moving black cloth screen. Male tsetse are also attracted to a moving vehicle but an effective method of catching them has not yet been devised. These methods are impractical because of the relatively small numbers caught, unless there are numerous catching points. Also many flies will be old and die quickly. Nets have been used to catch flies for want of a better method, but it is difficult not to damage the flies. Under laboratory conditions, it has been shown that flies caught from the bush do not live as long as those emerging from pupae in the laboratory. However, preliminary investigations into the senses and behaviour of tsetse flies have been initiated in the hope that attractants may produce a new catching technique.

An attractant might also be used to lure wild flies on to a chemo-sterilant in a field trap and thereby reduce the present problems of supply and treatment. This idea is purely speculative at present but if it could be made to work it might create a new problem — how to make the flies leave the vicinity of the attractant and re-disperse among the wild population.

Our present research and plans for a release trial on an island in Lake Kariba rely on the pupae collected by teams of supervised Africans searching natural larviposition sites in the vicinity of two camps in the Zambezi Valley. Over one million pupae have been collected in about three years but this is insufficient for an operational release programme. However, Dr. Phelps has shown that for various reasons doubling the number of teams at one camp does not double the number of pupae collected, and that reliance on this method would involve considerable expense due to erecting and maintaining new camps and roads.

Our present methods of collecting pupae or adult flies are therefore not practical for an operational sterile-male release programme. However, Azevedo with G. morsitans and Nash with G. austeni have been successful in developing and maintaining laboratory colonies. In addition, the Agricultural Research Council, in association with the United States Department of Agriculture, has begun to investigate the possibilities of large-scale field rearing.

The field investigations are following several research lines simultaneously. It would be ideal if a natural population and its breeding could be concentrated, and three separate schemes are being tried. In the first two schemes, cattle were either tethered at regular intervals to form a grid or were allowed to roam freely in a large paddock within favourable tsetse habitats of riverine-mopani woodland containing numerous larviposition sites, but few large indigenous animals. These trials each lasted for a year. The tsetse populations round the sites and adjacent control points that did not contain cattle permanently were sampled at weekly intervals by all-day catches of flies off tethered oxen. The results showed that a permanent food supply within the habitat is unlikely to concentrate significantly tsetse breeding. Several thousand flies were marked during these trials, and recaptures indicated extensive movement between the separate catching points.

Dr. Phelps' associated study of natural breeding sites showed that G. morsitans prefers to larviposit in dry, sandy river beds, under fallen trees and in the depths of ant-bear holes during the early, middle and late dry season months respectively. This discovery suggested a third method of concentrating the breeding of a natural population. A number of cement water pipes, 2 ft in diameter, have been placed near a river to simulate both fallen tree trunks and ant-bear hole sites. This project has just started and requires a full cycle of seasons to evaluate its possibilities. However, some pupae have been recovered from several of the pipes.

A large proportion of the Agricultural Research Council's field investigations is devoted to projects involving a variety of cage types. One type of wire-mesh cage, eight feet high, enclosing a quarter of an acre of the lower bush canopy, contains various small structures made of cement blocks with grass roofs offering additional settling sites and shelter for the tsetse flies. There are three of these large cages offering the fly extensive horizontal freedom in a relatively natural habitat. Another cage encloses a 30-feet-high evergreen tamarind tree so that the flies' vertical movements can be studied as they may be important for survival. These two types of cage design are subjected to very considerable ranges of temperature and humidity during the dry season.

A 6 ft ×6 ft ×8 ft cage made of cement-cavity bricks has a cement floor with a shaded plastic roof. A water-sprinkler system round the top of the walls increases humidity. The enclosed climatic conditions are more favourable for fly survival than field conditions. However, there is still a fairly considerable diurnal and seasonal variation.

A fourth project involves a 8 ft ×12 ft ×20 ft cage within a brick room maintained at 25°C, 70% humidity and a 12-h light cycle. A similar sized cage in the bush nearby is covered with reeds to exclude direct solar radiation but otherwise internal climatic conditions are similar to those recorded in the surrounding bush. The possibility of cage size being important for fly survival is being investigated by holding batches of flies in 8 in. × 8 in. × 11 in. cages within both these larger cages.

These different types of cage are being studied simultaneously. Fly survival is estimated at regular intervals by catches from bait oxen, by counting marked flies in the large cages, by counting the number of flies on certain areas of the cage walls or by counting the corpses in the smaller cages. Considerable developmental work has been necessary because of the difficulty in keeping the flies alive. Origin of the flies, predators

(especially ants), cage size and climatic conditions have been definitely identified as major factors in the high mortality rates, but other factors are probably present.

Oxen have mainly been used as the host animal so far and they appear to be satisfactory. However, an additional project has begun in which tsetse survival and reproduction under laboratory conditions will be compared using different hosts. Analysis of blood meals obtained by wild flies has shown that wart-hogs and bush-pigs give the majority feeds. These two animals are naturally relatively scarce compared with the abundant Impala antelope, which has never been reported as a tsetse host. The two pig species, Impala and buffalo have been captured and, with other indigenous animals when available, will be compared with oxen, sheep and guinea-pigs.

Laboratory and small field tests have therefore shown that there appear to be practical methods of sterilizing tsetse flies without seriously reducing their competitiveness and viability. Our major problem of rearing this insect in sufficiently large numbers is being actively investigated in both the field and laboratory.

ESSAI D'ELEVAGE DE Glossina fusca EN REPUBLIQUE CENTRAFRICAINE

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(Présenté par J. Itard)

Abstract — Résumé

TRIAL BREEDING OF Glossina fusca IN THE CENTRAL AFRICAN REPUBLIC. Breeding of Glossina fusca has been established in the Central African Republic both in natural and in artificial conditions, the latter in an air-conditioned room. This trial enabled some details of the biology of this species to be ascertained and an important fact to be confirmed, i.e. the very long pupal life, which can be more than 80 days, when the climatic conditions are unfavourable.

ESSAI D'ELEVAGE DE Glossina fusca EN REPUBLIQUE CENTRAFRICAINE. Un élevage de Glossina fusca a été réalisé à Bouar (République centrafricaine), dans des conditions naturelles, d'une part, et en salle climatisée, d'autre part. Cet essai d'élevage a permis de préciser certains points de la biologie de cette espèce. Il a permis en particulier de confirmer un fait important: la très longue durée de pupaison lorsque les conditions climatiques sont défavorables, cette durée pouvant dépasser 80 jours.

INTRODUCTION

L'élevage des glossines appartenant au groupe fusca n'a, semble-t-il, été tenté que par Jordan[1] qui, au Nigéria, avec une installation de fortune, est arrivé à élever G. tabaniformis, G. medicorum et G. fusca. Pour cette dernière espèce, la durée de la période pupale a été de 36,7 jours pour les mâles et de 33,9 jours pour les femelles, la durée moyenne de la vie des femelles étant de 30 jours.

Depuis 1960, à la station de Béwiti (République centrafricaine) les auteurs ont essayé de réaliser un élevage de Glossina fusca. Tous les essais ont été aussi décevants les uns que les autres, la survie des glossines n'excédant généralement pas quelques heures, au mieux quelques jours.

A la fin de 1965 les recherches ont été reprises, en élevant séparément chaque glossine en tube de Borrel. Un essai a été réalisé en conditions naturelles, en pleine forêt, à la station de Béwiti. Un deuxième vient d'être mis en route au mois de juin 1966: il est fait en salle climatisée, au Centre de recherches de Bouar.

[1] JORDAN, A.M., The ecology of the fusca group of tsetse flies (Glossina) in Southern Nigeria, Bull. ent. Res. 53 2 (1962) 355-85.

MATERIEL ET METHODES

A. Elevage en conditions naturelles

1. Installations

Pour bénéficier au maximum des conditions de température et d'hygrométrie dans lesquelles se trouvent naturellement les glossines, l'élevage a été installé en pleine forêt, en un endroit où les glossines abondent. Les installations étaient réduites au minimum: un simple abri en paille de 3 m × 3 m entouré d'une clôture de grillage, et une table métallique dont les pieds plongeaient dans des récipients contenant de l'huile minérale, pour empêcher les invasions de fourmis (malgré cette précaution l'élevage a été à plusieurs reprises détruit par ces insectes). Un thermo-hygromètre enregistreur était également installé dans l'abri.

2. Conditions climatiques

L'élevage a été commencé au mois de novembre 1965 et a été poursuivi jusqu'à la fin du mois de mars 1966, période correspondant à la saison sèche, qui, cette année, a été particulièrement sévère puisque aucune précipitation n'a été relevée entre le 7 novembre et le 14 mars. Des températures extrêmes de 35°C et de 10°C ont été relevées, l'hygrométrie variant entre 90 et 30%.

On doit noter que dans la nature, pendant les mois de février, mars et avril, les glossines disparaissent pratiquement.

3. Conditions d'élevage

Glossines adultes. Les glossines adultes sont élevées une par une en tube de Borrel, fermé par une toile moustiquaire à grosse maille. Dès leur capture, pour les glossines sauvages, ou le lendemain de leur naissance, pour celles nées en élevage, elles sont mises à gorger sur mouton ou sur chèvre. Par la suite, elles sont nourries tous les trois jours. Après chaque repas elles sont placées dans un nouveau tube stérilisé.

Les glossines nées en élevage sont mises avec un mâle le premier jour après leur naissance, puis séparées le troisième jour.

Pupes. Les pupes récoltées dans la nature ou obtenues en élevage artificiel sont placées dans des tubes de Borrel contenant un peu de sable sec, recouverts de toile moustiquaire. Les essais faits avec du sable humide ont été décevants, les tubes étant rapidement envahis par des moisissures.

B. Elevage en salle climatisée

L'élevage de Glossina fusca en salle climatisée a été entrepris à Bouar, au mois de juin 1966.

1. Installation

Le local d'élevage est constitué par une salle de 3 m × 3m. Une température de 23°C à 24°C y est maintenue par climatiseur contrôlé par un thermostat. L'humidité relative est maintenue à 80 - 90% par un humidificateur muni d'un hygrostat. L'aération est assurée par le ventilateur couplé avec le climatiseur.

2. Conditions d'élevage

Les glossines adultes et les pupes sont élevées dans des conditions identiques à celles de l'essai en plein air. La seule différence à signaler est que nous avons utilisé des lapins à la place des moutons ou des chèvres comme source de nourriture pour les glossines.

RESULTATS

A. Elevage en conditions naturelles

Glossines capturées dans la nature. De nombreux individus sont morts dans les jours suivant leur mise en élevage; cela semble pouvoir être imputé en grande partie à la précarité des installations et peut-être à la sévérité de la saison sèche. Dix glossines femelles ont vécu plus de 10 jours.

La durée moyenne de la vie a été de 68 jours, la durée maximale atteignant 112 jours.

Le nombre moyen de pupes pondues par femelle a été de 3, 1; 2 glossines ont pondu 5 pupes.

Une glossine a donné naissance à une deuxième pupe 4,5 jours après la première. Cette deuxième pupe était de petites dimensions. Une glossine mâle en est éclose après une pupaison de 71 jours.

Sans tenir compte de ce cas qui nous paraît aberrant, la période interlarvaire moyenne a été de 19,5 jours avec une durée minimale de 14 jours et une durée maximale de 27 jours.

L'élevage des mâles sauvages est beaucoup plus difficile que celui des femelles: ils ne survivent généralement pas plus de trois ou quatre jours.

Pupes d'élevage. Trente et une pupes ont été obtenues: 13 sont écloses, donnant 7 glossines femelles et 6 mâles.

Pour les femelles la durée moyenne de la pupaison a été de 72,5 jours (maximum 82 jours, minimum 52 jours).

Pour les mâles elle a été de 73,5 jours (maximum 85, minimum 55 jours).

On doit noter que les durées minimales de 52 et 55 jours ont été observées sur des glossines ayant éclos à la fin du mois de mars, soit après le retour des premières pluies.

Pupes sauvages. Plus de 200 pupes sauvages ont été mises en élevage, 54 ont éclos donnant naissance à 33 glossines femelles et 21 mâles. La durée maximale de la pupaison constatée a été de 62 jours

(ce chiffre n'a qu'une valeur indicative, car dans ce cas on ignore évidemment la date de la ponte).

Glossines de deuxième génération. Soixante-sept glossines sont nées en élevage (40 femelles et 27 mâles), 54 provenant de pupes récoltées dans la nature, 13 provenant de glossines élevées artificiellement.

Sur les 40 glossines femelles, 19 ont vécu moins de 10 jours, 17 entre 10 jours et 60 jours, 4 plus de 60 jours.

Sur les 27 mâles, 20 ont vécu moins de 10 jours, 2 plus de 60 jours.

Six glossines de deuxième génération ont donné naissance à 9 pupes.

La durée écoulée entre l'éclosion de la femelle et la ponte de la première larve a été en moyenne de 32 jours (extrêmes: 21 et 41 jours).

B. Elevage en salle climatisée

Les résultats ne sont encore que très fragmentaires et ne portent que sur un nombre très limité de glossines.

Onze glossines (3 mâles et 8 femelles) ont été placées dans la salle d'élevage.

Les trois mâles n'ont vécu que 8, 11 et 30 jours.

Trois femelles ont vécu plus de 100 jours. Les autres ont vécu 82, 54, 47, 33 et 13 jours.

Trois glossines n'ont jamais eu de descendance bien qu'elles aient vécu 54, 82 et plus de 100 jours. On peut supposer qu'elles n'ont pas été fécondées avant leur capture.

Les 4 autres ont pondu 13 pupes, dont 2, qui sont encore vivantes, ont pondu 5 et 4 pupes.

La période interpupale moyenne a été de 14 jours, avec des extrêmes de 21 et de 7 jours.

Quatre pupes ont éclos donnant 1 glossine femelle (pupaison, 40 jours) et 3 mâles (41, 41 et 42 jours). La femelle est morte après 28 jours, les mâles après 12, 3 et 1 jours.

Une pupe provenant de l'élevage de la station de Béwiti a été élevée dans la salle climatisée de Bouar. Elle a donné naissance à une glossine femelle après un délai de 33 jours. Au même moment, dans les conditions naturelles de Béwiti, cette durée dépassait en moyenne 72 jours avec une durée minimale de 55 jours.

POSSIBILITIES OF THE STERILE-MALE TECHNIQUE FOR THE CONTROL OF LIVESTOCK INSECTS IN THE UNITED STATES OF AMERICA

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Abstract

POSSIBILITIES OF THE STERILE-MALE TECHNIQUE FOR THE CONTROL OF LIVESTOCK INSECTS IN THE UNITED STATES OF AMERICA. Radiation and chemosterilant research on various livestock pests in the United States of America is reviewed from the standpoint of control by the sterile-male technique.

About five years ago, after the feasibility of the sterile-male technique of insect control was proved by the eradication of screw-worms, Cochliomyia hominivorax (Coquerel), from Curaçao and the south-eastern United States of America, the USDA Livestock Insects Investigations Group at Kerrville, Texas, turned its attention to the application of the sterility principle to other livestock insects. Progress in several such research projects was discussed in a recent review by Graham and Harris [1].

Frequently the initial attempt to use the sterile-male technique of control against a particular species has immediately called attention to a lack of fundamental knowledge about the arthropod's behaviour, life history and ecological relationships. Thus studies of gametogenesis, mating habits and dispersal in the field, and other types of biological investigation are often needed to support the experiments with the sterile-male technique. Such studies with livestock insects are currently under way in a number of state and federal laboratories in the United States of America and Canada. Also, a great deal of interest exists throughout North America in the concept of integrated control programmes or population suppression measures [2].

Several attempts have been made to eradicate cattle grubs, Hypoderma spp., by treating all the cattle in an isolated area with a systemic insecticide [3]. For example, tests in the Empire Valley of British Columbia and on Santa Rosa Island off the coast of California showed that populations of cattle grubs can be reduced by more than 99% by careful application of systemic insecticides, and an even more successful programme is currently in progress in Ireland [4]. Knipling [5] suggested that the release of a relatively small number of sterile male heel flies (adult cattle grubs) in a selected area might complete an eradication effort after systemic insecticides had achieved their maximum effect. Drummond [6] showed that gamma irradiation of pupae of Hypoderma lineatum (de Villers) with

1000 and 2500 R resulted in the production of male flies with reduced fertility: at a dose of 2500 R, female flies were completely sterile, and at 5000 and 7500 R both sexes were sterilized; also, irradiated females produced eggs that did not hatch, even when these females were mated to normal males, and although males irradiated at all levels were capable of sperm transfer, eggs from females mated to males treated with 5000 and 7500 R did not hatch. Drummond did not study the behaviour of irradiated heel flies outside the laboratory, but observations in the laboratory indicated that irradiated flies were normal in appearance and active, and behaved like unirradiated flies.

Weintraub [7] showed that sterile heel flies could be produced by exposing various stages of cattle grubs to a chemosterilant. He applied apholate, hempa, methiotepa, metepa, and 1,4-butane diol dimethane sulphate by (a) dipping puparia in sterilant solutions, (b) topical application of sterilant solutions to the thorax or abdomen of adults, (c) tarsal contact of flies with residual films of chemosterilants, or (d) dipping of newly hatched larvae in aqueous solutions of chemosterilant. The chemosterilants are apparently all somewhat toxic to the flies, but the most promising results (92-100% sterility) were obtained by dipping puparia in solutions of 3% metepa in acetone.

The horn fly, Haematobia irritans (L.), and the stable fly, Stomoxys calcitrans (L.), are probably the two most widely distributed biting flies that affect cattle in the United States of America, and both overwinter as pupae throughout much of the country. Cattle are therefore infested each spring by a relatively small number of adults that emerge from these overwintering pupae. Thus the concept of Knipling [8] of releasing laboratory-reared sterile insects to control newly established or emerging populations might be applicable to these species.

Since large numbers of sterile insects must be available to accomplish any programme of sterile-male release, a key requirement is a mass rearing method. Adequate methods of rearing the stable fly have been available for many years [9], but the first true laboratory colony of the horn fly was first reported as recently as 1962 by Harris [10]. The techniques developed by Harris have since been modified by Schmidt et al. [11] and used to rear horn flies in quantity.

In 1964, Lewis and Eddy [12] reported that both sexes of the horn fly could be sterilized by irradiating pupae with 5000 R of gamma-rays from a ^{60}Co source, and that when irradiated pupae were mixed with untreated pupae and the two were allowed to emerge together, the viability of the resulting eggs decreased. Unreported studies in 1966 by Hoffman and associates indicated that both pupae and newly emerged adults (< 24-h old) of the Kerrville laboratory horn fly colony could be sterilized with 3000 R of gamma-rays but that these flies were not fully competitive sexually. As part of these studies, they made a preliminary field release of sterile horn flies on a semi-isolated herd of cattle at Camp Stanley, Texas. Although cross-mating occurred (proved by non-viable eggs produced by captured flies), some viable eggs were also obtained during the six weeks of the study. In another phase of the same investigation, application of a non-residual insecticide and sterile-fly release were alternated. This combination appeared to be more promising than sterile-fly releases alone, particularly since control of biting flies by sterile-fly release is limited

by the number of such flies the host animal will tolerate without adverse effects.

Also at Kerrville, Harris and Frazar [13] demonstrated that apholate and tepa induce sterility in adult male and female horn flies when they are applied topically or mixed with the diet. However, mating tests indicated that sterilized males were not completely competitive with normal males.

Although we are unaware of any field trials with sterile stable flies, laboratory work has been conducted to establish their response to both ^{60}Co gamma irradiation and to chemosterilants. An unreported study by Harris and Frazar in 1962 demonstrated that both sexes of a laboratory colony of stable flies could be sterilized with 5000 R. Also, recent unpublished studies by Eschle and Dreiss at Kerrville showed that both sexes were sterilized with 3000 R but not with 1000 R. The sexual competitiveness of these flies is still being investigated.

In 1962, Harris [14] reported on sterility of the stable fly induced chemically with apholate, metepa and tepa. He found that apholate and metepa were approximately equal in effectiveness when they were applied topically to individual flies, and that tepa was slightly less effective. Also, exposure to a residual film of 10 mg of apholate for 48 h caused a 99% inhibition of egg hatch.

Drummond et al. [15] exposed unengorged and engorged nymphs and unengorged adults of the lone star tick, Amblyomma americanum (L.), to 250-7500 R of gamma irradiation and determined effects on engorgement and moulting of nymphs, and engorgement and egg laying of females and hatch of eggs. Most unengorged nymphs engorged normally, but those exposed to 2500 R or more did not moult to adults. Engorged nymphs exposed to 2500 R at one day after engorgement did not moult to adults, but engorged nymphs exposed to as much as 15 000 R at two weeks after engorging did moult. Irradiation of adults at 250 R had no effect on engorgement, egg laying and hatch of eggs. Some viable eggs were laid by females treated as unengorged nymphs or as nymphs engorged one day or one week, or treated as adults at 500 R, but no eggs were laid by those treated at 500 R as nymphs engorged two weeks; at 1000 R, females engorged normally but did not lay eggs. When males were treated at 500 R, some viable eggs were recovered, but results varied depending on the stage at which they were treated. At 2500 R or more, treatment of either sex prevented reproduction.

More recent studies (Drummond, unpublished) indicated that Amblyomma maculatum Koch, the Gulf Coast tick, responds to gamma irradiation in a manner similar to that of A. americanum. During the moulting period the ticks became more tolerant of the lethal effects of irradiation. Results were variable in these preliminary tests, but most females from nymphs treated at 860 and 2150 rads two and three weeks after engorging, and mated to normal males, engorged normally but did not lay eggs. Normal females mated to similarly treated males engorged normally and laid eggs, but hatch was prevented or greatly reduced.

Studies under way (Drummond, unpublished) on Rhipicephalus sanguineus (Latreille), the brown dog tick, indicate that, as with the Amblyomma species, both sexes are sterilized by gamma irradiation.

Incomplete studies by Graham and Price (unpublished) indicate that genetically sterile males of Boophilus can be produced by crossing the males of Boophilus annulatus (Say) with females of B. microplus (Canestrini)

and from the reverse cross. When the hybrid males from either cross were mated with females of either of the two species, the eggs produced by the females did not hatch.

All these studies with ticks have been of an exploratory or preliminary nature. Additional data on longevity, mating activity, sexual competitiveness of irradiated males and other factors must therefore be obtained before the sterile-male technique can be used to control ticks in the field.

REFERENCES

[1] GRAHAM, O.H., HARRIS, R.L., Bull. ent. Soc. Am. 12 (1966) 319.
[2] KNIPLING, E.F., Bull. ent. Soc. Am. 12 (1966) 7.
[3] GRAHAM, O.H., DRUMMOND, R.O., J. econ. Ent. (in press).
[4] THORNBERRY, H., J. Am. vet. med. Ass. 147 (1965) 1593.
[5] KNIPLING, E.F., USDA Rep. ARS-33-98 (1964).
[6] DRUMMOND, R.O., Int. J. Radiat. Biol. 7 (1963) 491.
[7] WEINTRAUB, J., Canada Agricultural Research Station, Lethbridge; personal communication.
[8] KNIPLING, E.F., J. econ. Ent. 48 (1955) 459.
[9] McGREGOR, W.S., DREISS, J.M., J. econ. Ent. 48 (1955) 327.
[10] HARRIS, R.L., Nature, Lond. 196 (1962) 91.
[11] SCHMIDT, C.D., HARRIS, R.L., HOFFMAN, R.A., Ann. ent. Soc. Am. 60 (1967) 508.
[12] LEWIS, L.F., EDDY, G.W., J. econ. Ent. 57 (1964) 275.
[13] HARRIS, R.L., FRAZAR, E.D., J. econ. Ent. 59 (1966) 1171.
[14] HARRIS, R.L., J. econ. Ent. 55 (1962) 882.
[15] DRUMMOND, R.O., MEDLEY, J.G., GRAHAM, O.H., Int. J. Radiat. Biol. 10 (1966) 183.

PREMIERS RESULTATS D'UN NOUVEL ESSAI D'ELEVAGE DE Glossina tachinoides West.

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Abstract — Résumé

PRELIMINARY RESULTS OF A NEW BREEDING EXPERIMENT IN Glossina tachinoides West. Results recorded at the beginning of this breeding experiment, which was carried out under more favourable conditions than the previous one, enabled the author to ascertain some fundamental characteristics of the biology of G. tachinoides in the laboratory.

PREMIERS RESULTATS D'UN NOUVEL ESSAI D'ELEVAGE DE Glossina tachinoides West. Les résultats obtenus par ce début d'élevage, réalisé dans de meilleures conditions que le premier essai, permettent de préciser certaines constantes fondamentales de la biologie de G. tachinoides au laboratoire.

INTRODUCTION

Les pupes récoltées pour cet élevage provenaient des gîtes de Riggil (rive camérounaise de Chari) les plus proches du laboratoire. Au total quatre récoltes, effectuées les 24 février, 19 et 29 mars et 15 avril dans le même gîte, ont permis de rapporter au laboratoire 930 pupes. Après un tri sous binoculaire éliminant les pupes défectueuses, on en a conservé 280 (tableau I, fig. 1).

La première remarque à faire est que pour le gîte considéré le maximum de pontes se situe, comme en 1963, aux mois de février et mars.

MATERIEL ET METHODES

Les pupes ainsi récoltées ont été placées dans de petits cristallisoirs, sur une mince couche de sable préalablement stérilisé. Chaque cristallisoir était «coiffé» d'une cage Roubaud et placé dans la pièce d'élevage.

a) Eclosions (tableau II)

Cent une pupes (280-158-19-2), soit 36,1% de celles placées dans les conditions d'élevage, n'ont pas éclos. A la dissection on note un fort pourcentage de mouches séchées en pupes ou mortes au moment de l'éclosion.

TABLEAU I. RECOLTE DES PUPES

Date des récoltes	Nombre de pupes récoltées	Nombre de pupes en élevage
24 février	150	12
19 mars	399	49
29 mars	338	209
15 avril	43	10

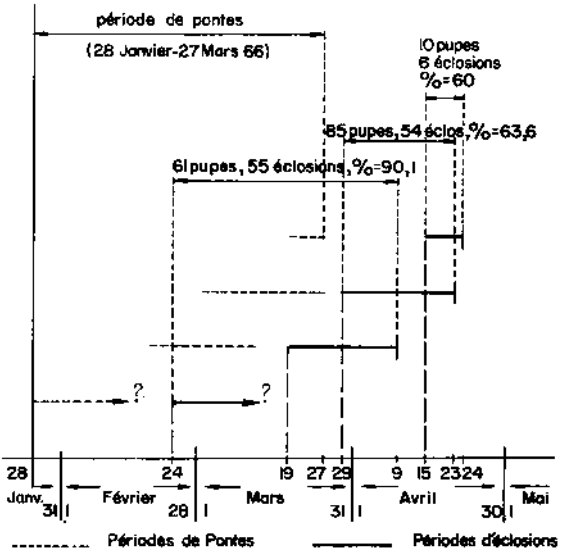

FIG. 1. Pontes et éclosions au laboratoire.

Deux Thyridanthrax argentifrons sont nés des pupes provenant des récoltes de février.

Contrairement à ce qui a été observé en 1963, il n'y a eu que 19 mouches non évoluées, soit 11,1% des éclosions totales. Le manque d'humidité pendant la pupaison apparaît donc bien comme un facteur fondamental.

Le lot de pupes du 29 mars a été divisé en trois groupes, chacun étant placé dans des conditions différentes.

A l'arrivée au laboratoire 54 pupes ont été mises au frigidaire à 5°C pendant 48 h avant d'être introduites dans la salle d'élevage. Cette série a donné un pourcentage d'éclosions de 70,4%. Cette pratique permet d'obtenir souvent un taux d'éclosion assez élevé.

TABLEAU II. ECLOSIONS

Date des récoltes de pupes	Nombre de pupes mises en élevage			Eclosions			Pourcentage d'éclosions		Mouches non évoluées	Thyridanthrax argentifrons A.
				Mâles	Femelles	Total				
24 février 19 mars		12 49	61	21	34	55	90,1		6	2
29 mars	Frigidaire	54		14	24	38	70,4		4	-
	Pièce d'élevage	85	209	22	32	54	63,6	46,4	5	-
	Salle temp. et hum. amb.	70		2	3	5	7,1		embryons désséchés	
15 avril			10	1	5	6	60		4	-
Totaux			280	60	98	158	56,4		19	2

Soixante-dix pupes ont été conservées sans précautions, dans un local à température voisine de 30°C et où l'humidité n'excédait jamais 15%. Cinq éclosions ont eu lieu au cours des deux premiers jours; l'évolution de toutes les autres pupes a été définitivement stoppée par la dessiccation.

Les 85 pupes placées dans des conditions normales d'élevage ont éclos à 63%.

Pour l'ensemble des récoltes, le pourcentage d'éclosions est de 56,4%.

b) Elevage des mouches

Les éclosions ont donné naissance à 60 mâles et 98 femelles, soit un total de 158 mouches qui ont ainsi pu être mises en élevage. Quelle que soit la période de récolte des pupes, le nombre de femelles est toujours supérieur à celui des mâles. Sur le total des éclosions 38% sont des mâles et 62% des femelles; il y a donc 1,63 fois plus de femelles que de mâles parmi les mouches normalement constituées.

Accouplement. Après éclosion et séparation des sexes les accouplements ont été réalisés de telle sorte que chaque femelle fécondée reste isolée dans une cage, ceci afin de suivre la destinée de chacune d'elle.

Les accouplements peuvent s'effectuer dès le premier jour, même avant le premier repas de sang; ils durent une heure et demie. Il a été observé qu'un mâle introduit dans la cage d'une femelle déjà fécondée ne tentait jamais de l'approcher. De plus, placé dans de telles conditions, il était presque toujours retrouvé mort le lendemain du jour d'introduction.

Repas. Les repas de sang ont tout d'abord été donnés sur l'homme en raison du petit nombre de mouches au début de l'élevage. Une tentative d'alimentation sur cobaye a dû être abandonnée; toutes les mouches nourries ainsi mouraient dans un délai maximal de trois jours. Il faut remarquer à ce sujet qu'une épidémie de pneumonie à virus sévissait à cette époque sur l'élevage des cobayes du laboratoire. L'étude des rapports entre cette maladie et le décès des mouches sera repris ultérieurement.

La chèvre, finalement, a été adoptée comme «source de nourriture» pour poursuivre l'élevage. Les mouches prennent ainsi un repas quotidien 6 jours sur 7.

Les mouches pondeuses refusent en général tout repas pendant les 2 ou 3 jours qui précèdent la ponte; par contre elles prennent habituellement un repas copieux dans la journée qui la suit.

Lumière. L'éclairage artificiel a été maintenu dans la salle d'élevage selon un rythme identique à celui observé à l'extérieur.

RESULTATS

a) Longévité des mouches

Placées dans de telles conditions, les cages étant changées chaque jour, un effectif voisin de 40 mouches a pu être maintenu pendant plusieurs semaines (fig. 2 et 3).

D'une manière générale, il apparaît que les mâles supportent moins bien la captivité que les femelles. La longévité moyenne observée est de 8,7 jours pour les mâles contre 22,8 jours pour les femelles.

La mortalité est toujours très importante le premier jour; les maximums de longévité observés ont été de 36 jours pour un mâle et 142 jours pour une femelle; 5 femelles seulement ont dépassé 100 jours de vie.

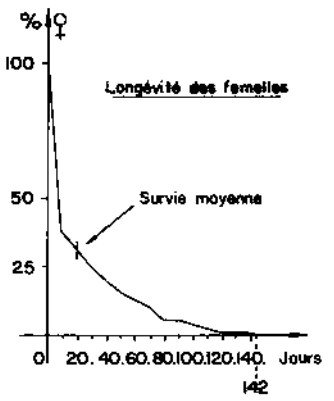

FIG. 2. Longévité des femelles.

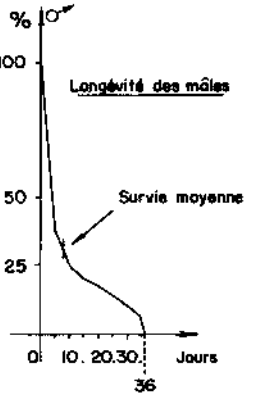

FIG. 3. Longévité des mâles.

b) Reproduction

Ponte. La mortalité des femelles étant importante dans les premiers jours de la vie (plus de la moitié) et toutes les femelles n'ayant pas été accouplées, seules ont été suivies celles à accouplement contrôlé et à ponte régulière. Ainsi 15 pondeuses ont été isolées et les pontes de chacune regroupées.

Ces 15 mouches ont pondu au total 96 larves, ce qui fait une moyenne de 6,4 larves par mouche, les maximums observés étant de 15 et 11 larves.

Ainsi que cela a été constaté dans différents élevages, les dernières larves pondues par une mouche sont souvent naines.

Premières gestations. La plupart des mouches montrent une durée de première gestation comprise entre 15 et 19 jours; sur les 15 mouches examinées, toutes les valeurs intermédiaires ont été remarquées et la durée moyenne de gestation est de 17 jours.

Intervalle entre les pontes. Sur 70 périodes interlarvaires observées l'intervalle moyen qui sépare deux pontes successives d'une même mouche est de 8,43 jours. Il faut donc une longévité d'au moins 93 jours pour qu'une mouche ponde 10 larves.

Des irrégularités dans les délais successifs de ponte ont été quelquefois observées, mais une régulation semble assurer le maintien du rythme; à une période interlarvaire courte en succède une plus longue.

Par contre, les intervalles apparaissent prolongés chez les vieilles mouches ayant pondu plus de 10 larves. Le nombre de 15 larves pondues par une mouche paraît être un maximum.

Après sa dernière ponte, la mouche ayant vécu 142 jours n'avait pas de larves en formation.

Pupaison. L'étude précise des durées de pupaison a porté sur l'évolution des larves pondues au laboratoire. De l'examen précis de 53 pupaisons il est permis de conclure qu'à 25°C et avec 65 à 70% d'humidité relative la durée moyenne est de 31,6 jours.

Deux maximums de 46 et un minimum de 26 jours ont pu être observés.

BIBLIOGRAPHIE

GRUVEL, J., BALIS, J., Essai d'élevage de Glossina tachinoides W. au laboratoire, Revue Elev. Méd. vét. Pays trop. 19 1 (1966) 21-28.

ITARD, J., MAILLOT, L., Notes sur un élevage de glossines entrepris à partir de pupes expédiées d'Afrique à Maisons-Alfort, France, Revue Elev. Méd. vét. Pays trop. 19 1 (1966) 29-44.

ITARD, J., MAILLOT, L., Elevage en France de G. m. morsitans W. et G. tachinoides W., C.r. hebd. Séanc. Acad. Sci., Paris 261 12 (1965) 5626-28.

CURRENT STATUS OF SCREW-WORM ERADICATION IN THE SOUTH-WESTERN UNITED STATES OF AMERICA AND THE SUPPORTING RESEARCH PROGRAMME

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Abstract

CURRENT STATUS OF SCREW-WORM ERADICATION IN THE SOUTH-WESTERN UNITED STATES OF AMERICA AND THE SUPPORTING RESEARCH PROGRAMME. The supporting research programme on screw-worm eradication in the south-western United States of America is described, the emphasis being on the current need to integrate into the fly release programme a thorough knowledge of factors not previously encountered.

The successful use of artificially reared and irradiated screw-worm flies, Cochliomyia hominivorax (Coquerel), to eradicate indigenous populations of this livestock pest from the United States of America is well documented [1]. Since Knipling [2] first proposed the use of irradiated males of this species to reduce wild populations, screw-worms have been eradicated from the south-eastern states (1959), from Texas and New Mexico (1964) and from Arizona and California (1965). The states north and east of these four south-western states remain free of screw-worm infestations, but reinfestations continue to be an intermittent problem in the states adjacent to Mexico.

Although refinements in techniques have improved the efficiency of methods of rearing, irradiating and handling various life stages of the screw-worm, the basic procedure has not changed since the first major effort at eradication in Florida. For the sequence of the development of present techniques, see Refs [3-6]; also, an excellent recent review has been compiled by Baumhover et al. [7].

Unfortunately, progress in production of irradiated flies has not been matched by knowledge of the population dynamics, dispersal patterns and behavioural characteristics of wild populations in the area where sterile flies are currently being released. Thus, to protect the United States of America from reinfestation, it is necessary to maintain a control zone in northern Mexico 1500 miles long and 200-300 miles in depth, and the continuous release of an adequate number of sterile flies over this vast area is at present beyond the capability of the programme.

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The area includes mountainous terrain and deep canyons that prevent the use of previously established swath widths and altitudes of release, and encompasses life zones ranging from tropical rain forests continuously infested with screw-worms to rarely infested subtropical desert. Invariable grid patterns with a uniform rate of release of sterile males per square mile are therefore no longer applicable.

The present review is limited to the status of the supporting research programme on screw-worm eradication in the south-western United States of America, with emphasis on the current need to integrate into the fly release programme a thorough knowledge of factors not previously encountered.

I. SEASONAL ABUNDANCE OF SCREW-WORMS IN MEXICO

Although populations of screw-worm flies are present throughout the five northern states of Mexico that border the United States of America, the seasonal weather cycles fortunately limit them sufficiently so that simultaneous increases do not occur throughout the whole zone.

In the high plateau regions of Chihuahua and Coahuila, cold weather is a critical factor, and the weather during the spring also prevents populations from reaching outbreak proportions until the summer rains come in July and August. Thus, screw-worm fly activity in this area reaches a peak during September and October, and it is possible to leave a large portion of this zone untreated until early summer. Along the east and west coasts of the northernmost Mexican states screw-worms occur throughout the year, and populations normally increase during the early spring months. However, in these regions hot, dry summer weather is a major limiting factor, and infestations usually decline during late July, August and early September.

The mapping of the limits of the two zones by Hightower et al. [8] has now permitted us to increase releases of sterile flies near the potential outbreak centres and to cease releases where conditions are unfavourable to build-up of population. However, lack of detailed information on the effects of weather and other environmental factors on screw-worm populations in Mexico has prevented precise timing of these shifts to ensure maximum effects from the released flies.

Davis [9] recently proposed a method of reducing the data on time-space distribution of local populations of screw-worm flies to contour maps. If maps of these profiles of local populations at given points in time can be correlated with sufficient data on the various factors known to affect populations, it may be possible to delineate more accurately areas where population increases are most likely to occur. Priorities for releases are now established while population levels are still low enough to be controlled with minimum releases. However, the rapid means of communication that enabled livestock growers in the south-eastern and south-western United States of America to report quickly populations of screw-worms are not available in many parts of Mexico, and the response to reports of infestations is also delayed. More precise methods of determining the extent and level of infestations in Mexico are therefore required to offset the disadvantage.

II. SWATH WIDTHS

During the eradication programme in the south-eastern United States of America, sterile flies were released over infested areas in parallel lines two miles apart. Since the lines were shifted by one mile each week, an effective coverage in one-mile swaths was achieved within each two-week period. Essentially the same procedure was followed during the eradication programme in Texas. Persistent infestations, isolated populations and watercourses received special treatment, but the basic pattern of release remained the same. The same swath width is neither a practical nor a possible basic release pattern in the control zone in Mexico. Instead, flight lanes four to eight miles apart with weekly offsets of two miles are currently being used with some success to achieve a swath width of two to four miles respectively every two weeks in areas where we have sufficient information about the seasonal fluctuations in screw-worm populations. Releases made at the rate of 400 flies per square mile on invariable flight lanes eight and 12 miles apart are not effective in preventing population increases when weather is favourable to screw-worm fly activity [10]; however, they are more effective when wild populations are low. Also, releases made at the rate of 1000 flies per square mile per week on flight lanes 12 miles apart with weekly offsets of six miles to achieve a swath width of six miles every two weeks are not effective in reversing a rising trend in populations.

The effects of native flies entering the treated areas from adjacent untreated areas could not be ascertained in the tests upon which these conclusions are based. However, wide swath widths (as much as four miles) can be used effectively when local populations are low and scattered, and the incidence of screw-worms in Mexico is generally lower and more patchy than that in Texas or Florida before eradication.

III. STRAINS OF FLIES

A strain of screw-worm flies derived from collections in Florida and Georgia by Baumhover (unpublished data) was used successfully during the eradication campaigns in the south-east and south-west of the United States of America. This strain has been reared in highly artificial conditions (i.e. the colony was maintained in continuous darkness) for more than 100 generations. Since behavioural changes had occurred in other species after prolonged colonization [11, 12], multiple collections of egg masses and pupae were made along the east and west coasts of Mexico during 1965 to derive a new strain of flies that might possibly be more suitable for release in Mexico. This strain is currently being used in Mexico despite the results of recent laboratory tests in which males of the Mexico strain were not as sexually active as males of the Florida strain; the finding may not be the conclusive determinant of the effectiveness of the flies in field releases [13].

Although other strains of flies selected for visible genetic markers and resistance to starvation are being maintained in laboratory cultures, the use of these strains for population control must await the development of more sophisticated techniques for assessing their suitability for the purpose.

IV. ATTRACTANTS AND CHEMOSTERILANTS

The potential usefulness of an attractant-chemosterilant combination as a method of holding a native population of screw-worms at a low level has been recognized for a number of years. The possibility is particularly attractive as a partial or complete replacement for sterile-fly release in a barrier zone operation such as the one proposed for the Isthmus of Tehuantepec in southern Mexico. Also, a highly efficient attractant would be extremely useful for surveys conducted before sterile-fly releases and for the detection of low-level infestation in the final phases of an eradication programme. Fletcher and associates at Mission, Texas, have tested a large number of natural and synthetic materials in the laboratory, in outdoor cages and in the field, but they have not yet discovered a substance that is consistently equal to decomposing liver as a bait in the standard traps operated by Parman [14] more than 30 years ago. However, Fletcher et al. [15] showed that a pheromone that elicits a mating response in unmated female flies can be recovered from male screw-worm flies.

Knipling [16] pointed out the advantages of exposing a population of insects to a chemosterilant rather than to a toxicant to produce sexually sterile individuals that would neutralize the reproductive capacity of other individuals by mating with them. Crystal [17] evaluated hundreds of preselected compounds as chemosterilants for the screw-worm in the laboratory, and reported three that can be used to produce male sterility without apparent adverse effects on longevity, vigour or mating competitiveness. It remains to be seen whether these chemicals can be used safely outdoors, but the probability is excellent that if an efficient attractant does become available, a useful chemosterilant will also be available.

REFERENCES

[1] BAUMHOVER, A.H., J. Am. med. Ass. 166 (1966) 240.
[2] KNIPLING, E.F., J. econ. Ent. 48 (1955) 459.
[3] BUSHLAND, R.C., HOPKINS, D.E., J. econ. Ent. 46 (1953) 648.
[4] BAUMHOVER, A.H., GRAHAM, A.J., HOPKINS, D.E., DUDLEY, F.H., NEW, W.D., BUSHLAND, R.C., J. econ. Ent. 48 (1955) 462.
[5] GRAHAM, A.J., DUDLEY, F.H., J. econ. Ent. 52 (1959) 1006.
[6] SMITH, C.L., J. econ. Ent. 53 (1960) 1110.
[7] BAUMHOVER, A.H., HUSMAN, C.N., GRAHAM, A.J., Screw-worms, in Insect Colonization and Mass Production, (SMITH, C.N. Ed.), Academic Press, New York (1966) 533.
[8] HIGHTOWER, B.G., DAVIS, R.B., BAUMHOVER, A.H., GRAHAM, O.H., J. econ. Ent. 59 (1966) 416.
[9] DAVIS, R.B., Ecology (1967) (in press).
[10] DAVIS, R.B., HIGHTOWER, B.G., ALLEY, D.A., TURNER, J.E., LOPEZ, E. (in preparation).
[11] DAME, D.A., WOODARD, D.B., FORD, H.R., WEIDHAAS, D.E., Mosquito News 24 (1964) 6.
[12] FYE, R.L., LA BRECQUE, G.C., J. econ. Ent. 59 (1966) 538.
[13] SPATES, G.E., HIGHTOWER, B.G., J. econ. Ent. (1967) (in press).
[14] PARMAN, D.C., J. econ. Ent. 38 (1945) 66.
[15] FLETCHER, L.W., O'GRADY, J.J., Jr., CLABORN, H.V., GRAHAM, O.H., J. econ. Ent. 59 (1966) 142.
[16] KNIPLING, E.F., J. econ. Ent. 55 (1962) 782.
[17] CRYSTAL, M.M., J. med. Ent. 2 3 (1965) 317.

RECHERCHES SUR LES CHROMOSOMES DE GLOSSINES

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Abstract — Résumé

RESEARCH ON THE CHROMOSOMES OF GLOSSINA. The somatic chromosomes have been made evident from the peri-oesophageal brain and the Malpighian tubes of the 24-hour-old pupa of Glossina tachinoides West. (2n = 6), and Glossina morsitans morsitans West. (2n = 10).

RECHERCHES SUR LES CHROMOSOMES DE GLOSSINES. A partir de la masse nerveuse péri-œsophagienne et des tubes de Malpighi de la pupe âgée de 24 h, les chromosomes somatiques ont été mis en évidence chez Glossina tachinoides West. (2n = 6), et chez Glossina morsitans morsitans West. (2n = 10).

INTRODUCTION

Dans le cadre des recherches sur l'action des radiations ionisantes chez les insectes du genre Glossina, nous avons entrepris une étude sur les chromosomes des glossines élevées au laboratoire d'entomologie de l'IEMVT.

Nous exposons ici les premiers résultats obtenus.

MATERIEL ET METHODES

Les études ont porté sur Glossina tachinoides West., originaire du Tchad, et sur Glossina morsitans morsitans West. (Glossina morsitans orientalis Vand.), originaire de Rhodésie. Nous avons également entrepris des recherches sur le matériel chromosomique d'une troisième espèce, Glossina austeni, dont les pupes nous ont été obligemment fournies par le Dr Nash[1]. Les résultats, chez cette dernière espèce, ne sont cependant pas suffisamment nets pour pouvoir être exposés ici.

La recherche du matériel chromosomique a été effectuée chez la pupe âgée de 24 h. Chez l'insecte adulte, la recherche des chromosomes, en particulier dans les cellules sexuelles, a, jusqu'ici, été décevante.

La technique préparatoire suivie s'inspire des travaux publiés par Breland. Les pupes âgées de 24 h sont disséquées sous la loupe binoculaire, dans une ou deux gouttes de sérum physiologique. L'enveloppe pupale est fendue au rasoir, dans le sens longitudinal, et le contenu interne de la pupe isolé.

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Au cours des dissections suivantes, il est nécessaire de transférer plusieurs fois les tissus sur des lames propres, et de renouveler fréquemment le sérum physiologique, afin d'éliminer les globules graisseux toujours très abondants qui obscurcissent la préparation. A l'aide de fines aiguilles montées, on isole le tractus digestif en totalité. A l'extrémité antérieure, au niveau de l'œsophage, se trouve la masse nerveuse aisément repérable. Celle-ci est isolée, divisée en deux ou trois parties, et chacune d'elles est transférée dans une lame à concavité contenant de l'orcéine acétolactique dont la composition est la suivante: eau distillée: 33 ml; acide lactique: 33 ml; acide acétique: 33 ml; orcéine: 2 g.

La concavité est recouverte d'une lamelle afin de limiter l'évaporation du colorant, et les tissus sont laissés au contact du colorant pendant 3 h.

On procède de même pour les tubes de Malpighi, après les avoir séparés de l'intestin.

Les tissus sont ensuite transférés sur une lame propre contenant une goutte d'acide acétique à 50%, et la préparation est abondamment rincée avec cet acide. Après orientation au centre de la lame, le matériel est recouvert d'une lamelle et écrasé entre deux couches de papier filtre. La pression doit être assez élevée pour bien étaler la préparation et obtenir, si possible, une couche monocellulaire. Il faut veiller à ce que la lamelle ne soit pas déplacée au cours de cette opération, faute de quoi la préparation serait illisible. Lorsque les tissus sont bien étalés, les bulles d'air sont éliminées en ajoutant une goutte d'acide acétique au bord de la lamelle, puis celle-ci est scellée avec du vernis à ongle incolore. Les préparations ainsi obtenues peuvent être conservées un mois au réfrigérateur.

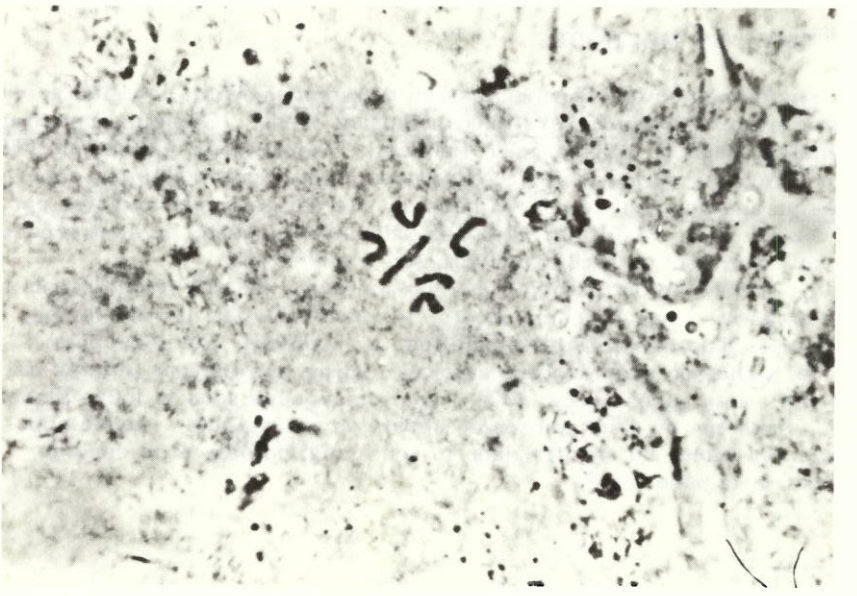

FIG. 1. Chromosomes de G. tachinoides (2n = 6). Début de la métaphase.

Les lames sont examinées à l'objectif à immersion, au microscope à contraste de phase. Les chromosomes apparaissent ainsi nettement et peuvent être aisément photographiés.

RESULTATS

Les figures de mitoses les plus nombreuses sont généralement obtenues avec les préparations effectuées à partir de la masse nerveuse péri-œsophagienne. Les tubes de Malpighi fournissent également de belles images, mais beaucoup plus rares. Nous avons obtenu quelques figures de mitose à partir des disques imaginaux situés à l'extrémité antérieure des diverticules trachéaux. Chez une pupe âgée de quelques jours, nous avons pu également mettre en évidence des chromosomes à partir des pattes qui étaient déjà parfaitement formées.

Nous n'avons pas, jusqu'à présent, pu repérer les glandes salivaires, ni les cellules germinales, et n'avons par conséquent pu mettre en évidence ni les chromosomes géants ni les chromosomes haploïdes.

Chez Glossina tachinoides, le nombre somatique de chromosomes est de 6 (fig. 1). Ces chromosomes, dont la longueur atteint 5 à 6 μm, présentent une morphologie identique et des différences de taille insignifiantes.

Chez Glossina morsitans morsitans, le nombre somatique de chromosomes est de 10 (fig. 2). Les chromosomes se répartissent en deux groupes: 6 chromosomes de taille relativement grande (6 à 7 μm environ) et 4 chromosomes très petits (1 à 2 μm).

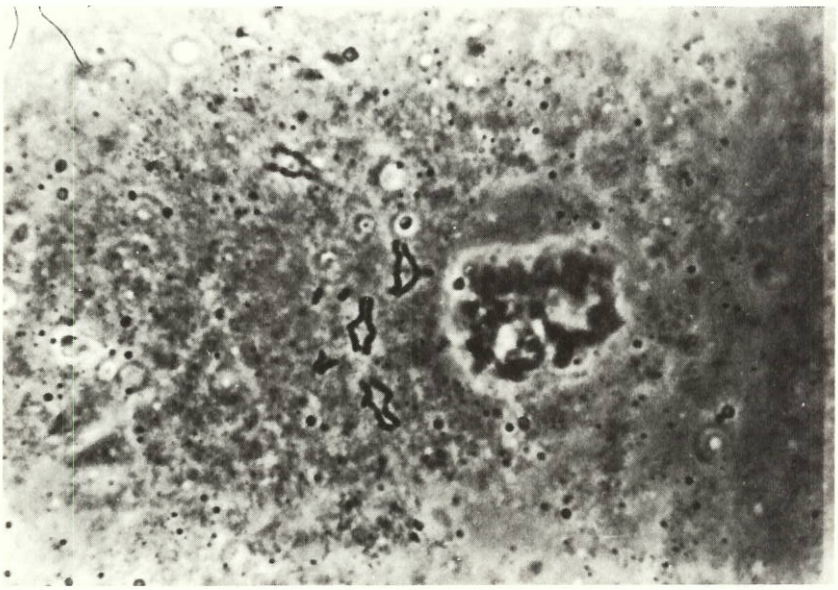

FIG. 2. Chromosomes de G. morsitans (2n = 10). Fin de la prophase, appariement des chromosomes homologues.

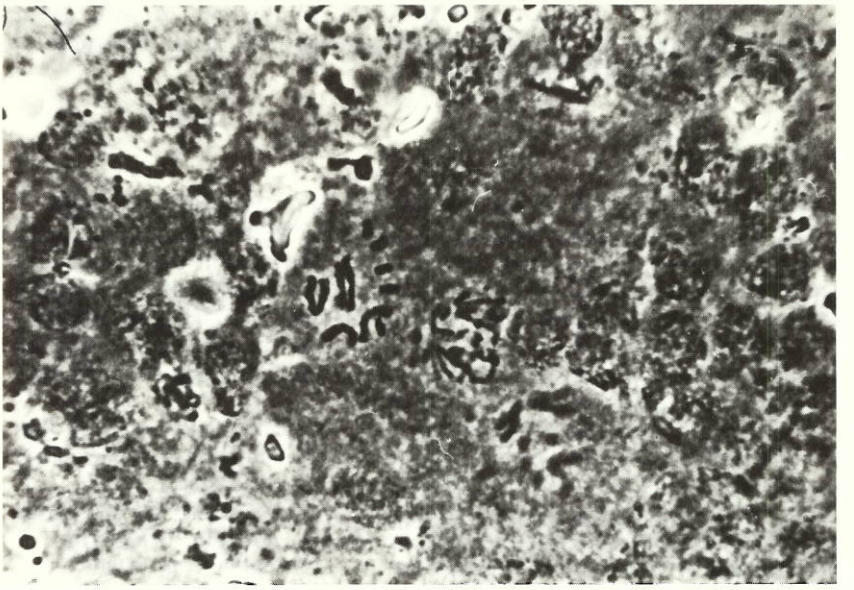

FIG. 3. Chromosomes de G. morsitans (pupe abortive, 2n = 11).

Chez une pupe abortive appartenant à cette dernière espèce, il a été mis en évidence 11 chromosomes (6 grands et 5 petits) (fig. 3). Il s'agit probablement d'un cas de polysomie, le sujet ayant 2n + 1 chromosomes.

BIBLIOGRAPHIE

BRELAND, O.P., Tex. J. Sci. 9 (1959) 183-90.
BRELAND, O.P., Ann. ent. Soc. Am. 54 3 (1961) 360-75.
ITARD, J., MAILLOT, L., C.r. hebd. Séanc. Acad. Sci., Paris 261 (1965) 5626.
ITARD, J., MAILLOT, L., Revue Elev. Méd. vét. Pays trop. 19 1 (1966) 29-44.

PREMIERS RESULTATS D'UN ESSAI D'IRRADIATION GAMMA SUR DES PUPES ET DES MALES ADULTES DE Glossina morsitans morsitans

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Abstract — Résumé

PRELIMINARY RESULTS OF A GAMMA-IRRADIATION TEST ON PUPAE AND ADULT MALES OF Glossina morsitans morsitans. Pupae of Glossina morsitans morsitans of various ages and 24-hour-old males have been exposed to different irradiation doses (^{137}Cs). Results presented show the percentage of hatchings and the fertility of adults from irradiated pupae, and the survival and fertility of irradiated adult males.

PREMIERS RESULTATS D'UN ESSAI D'IRRADIATION GAMMA SUR DES PUPES ET DES MALES ADULTES DE Glossina morsitans morsitans. Des pupes d'âges divers et des mâles âgés de 24 h de G. morsitans morsitans ont été soumis à différentes doses d'irradiation (^{137}Cs). Les résultats qui figurent dans le mémoire concernent les pourcentages d'éclosions et la fertilité des adultes issus des pupes irradiées, et la survie et la fertilité des mâles adultes irradiés.

Au début de décembre 1966 nous avons irradié un lot de pupes d'âges divers et 22 mâles âgés de 24 h appartenant à l'espèce Glossina morsitans morsitans, élevée au laboratoire d'entomologie de l'IEMVT à partir de pupes importées de Rhodésie en 1965.

1. IRRADIATIONS

Celles-ci ont été effectuées avec un irradiateur au césium-137 de 155 000 Ci, au Centre d'études nucléaires de Saclay. La dosimétrie a été effectuée, pour chaque exposition, par lecture au spectrophotomètre de tubes de sulfate ferreux adjoints à chaque lot. La précision obtenue était très satisfaisante, l'indétermination étant inférieure à 5% et, le plus souvent, 2%.

2. PUPES

Quatre-vingt-dix pupes âgées, au moment de l'irradiation, de 4 à 24 jours et réparties en deux classes (0 à 12 j; 13 à 24 j) ont été soumises à des doses comprises entre 4500 et 13 200 rad (tableau I). En outre, 13 pupes témoins ont été transportées dans les mêmes conditions que les pupes soumises à irradiation.

TABLEAU I. ECLOSIONS DE PUPES IRRADIEES
Classes d'âge: 0 à 12 j et 13 à 24 j

Dose (rad)	Nombre de pupes	Age à l'irradiation (j)	Nombre de pupes écloses et sexe de l'imago	Nombre de pupes non écloses ou mortes à l'éclosion	Total	
4500 4500	6 8	0 - 12 13 - 24	1 ♂ 8 (5 ♂+ 3♀)	5 0	14	Très significatif S = 1%
7500 7500	12 15	0 - 12 13 - 24	0 12 (4 ♂+ 8♀)	12 3	27	Très hautement significatif S = 1‰
8800 8800	6 8	0 - 12 13 - 24	0 7 (1 ♂+ 6♀)	6 1	14	Très significatif S = 1%
9300 9300	5 6	0 - 12 13 - 24	1 ♂ 6 (2 ♂+ 4♀)	4 0	11	Très significatif S = 1%
11000 11000	5 7	0 - 12 13 - 24	0 6 (2 ♂+ 4♀)	5 1	12	Très significatif S = 1%
13200 13200	5 7	0 - 12 13 - 24	0 3 ♂	5 4	12	Peu significatif S = 5%

Il existe une liaison très significative entre les pourcentages d'éclosions et l'âge des pupes, pour les doses allant de 4500 à 11 000 rad. Cette différence est peu significative à la dose de 13 200 rad. On peut supposer que seules les pupes de plus de 18 jours résistent à cette dose. Si l'on adopte, pour cette dose, les classes d'âge de 0 à 18 j et de 19 à 24 j, on obtient en effet les résultats figurant au tableau II.

La majorité des pupes non écloses étaient mortes peu de jours avant l'éclosion.

Toutes les pupes témoins transportées dans les mêmes conditions ont éclos. Ces résultats confirment ceux obtenus par le Dr Dean et Miss Wortham (Agricultural Research Council of Central Africa - Annual report for 1965).

Les femelles issues de pupes irradiées, accouplées avec des mâles normaux, n'ont à l'heure actuelle donné aucune pupe. Les femelles normales accouplées avec les mâles issus des pupes irradiées à 4500 rad ont donné chacune une pupe, en 30 jours.

Deux mâles issus de pupes irradiées, l'une à 7500 rad, l'autre à 9300 rad, accouplés avec des femelles normales, ont fécondé ces femelles, qui ont fourni chacune une pupe. Aucun des autres mâles irradiés n'a fécondé les femelles normales avec lesquelles ils avaient été accouplés.

Des pupes témoins sont issus huit femelles et cinq mâles qui, accouplés avec des imagos normaux, ont donné à ce jour huit pupes.

3. MALES AGES DE 24 h

Nous avons constitué cinq lots de mâles âgés de 24 h, répartis de la façon suivante:

5 ♂ témoins non transportés
3 ♂ témoins transportés dans les mêmes conditions que les mâles irradiés
5 ♂ irradiés à 13 500 rad
10 ♂ irradiés à 20 000 rad
7 ♂ irradiés à 25 000 rad.

Quarante jours après l'irradiation, on obtient les pourcentages de survie suivants:

Mâles témoins non transportés: 3/5, soit 60%
Mâles témoins transportés : 1/3, soit 33%
Mâles irradiés à 13 5000 rad : 2/5, soit 40%
Mâles irradiés à 20 000 rad : 1/5, soit 20%
Mâles irradiés à 25 000 rad : 0/5, soit 0%.

La moyenne de vie des mâles irradiés à 25 000 rad a été de 29 jours, avec un maximum de 36 jours. Tous les mâles ont été accouplés à l'âge de 7 jours, avec des femelles normales âgées de 3 jours. Les pupes produites 40 jours après l'irradiation se répartissent de la façon suivante:

Mâles témoins non transportés: 10 pupes pour 5 femelles, soit 2 pupes/femelle
Mâles témoins transportés: 9 pupes pour 3 femelles, soit 3 pupes/femelle

TABLEAU II. ECLOSIONS DE PUPES IRRADIEES
Classes d'âge: 0 à 18 j et 19 à 24 j

Dose (rad)	Nombre de pupes	Age à l'irradiation (j)	Nombre de pupes écloses	Nombre de pupes non écloses ou mortes à l'éclosion	Total	
13200	8	0 - 18	0	8	12	Très significatif S = 1%
13200	4	19 - 24	3	1		

Mâles irradiés à 13 500 rad:	3 pupes (dont 2 «abortives») pour 5 femelles, soit 0,6 pupe/femelle
Mâles irradiés à 20 000 rad:	1 pupe (pondue le 34^{e} jour) pour 10 femelles, soit 0,1 pupe/femelle
Mâles irradiés à 25 000 rad:	0 pupe pour 7 femelles.

Les mâles survivants ont été réaccouplés, à l'âge de 30 jours, avec des femelles normales et vierges, âgées de 3 jours. Tous les mâles se sont accouplés avec les femelles qui leur ont été présentées. Il n'y a pas eu diminution de la vigueur sexuelle des mâles irradiés par rapport aux témoins.

A ce jour, soit 8 jours après la date de réaccouplement, aucune des femelles n'a produit de pupes, pas plus les femelles accouplées avec des mâles irradiés que les femelles accouplées avec les mâles témoins, mais cette expérimentation a été effectuée depuis trop peu de temps pour que les premières pupes aient pu être pondues.

En résumé, nous pouvons noter que, chez les pupes âgées de plus de 12 jours irradiées à des doses comprises entre 4500 et 7500 rad, le pourcentage d'éclosions est compris entre 80% et 100%, ce qui est voisin des taux d'éclosion obtenus avec des pupes normales d'élevage. Quelle que soit la dose d'irradiation, les femelles issues des pupes irradiées sont stériles; chez les mâles on n'obtient par contre une stérilité totale qu'à des doses supérieures à 9300 rad.

L'irradiation des mâles adultes semble provoquer la stérilité totale à des doses supérieures à 20 000 rad, tout en permettant une survie dépassant en moyenne 30 jours.

4. CONCLUSIONS

Ces premières expérimentations ont été réalisées avec de faibles effectifs. Elles avaient surtout pour but de rechercher quelles étaient les doses à appliquer en fonction de stade de l'insecte et de l'âge pour obtenir la stérilité sans pour autant diminuer la longévité.

Les résultats obtenus avec les pupes sont en accord avec les résultats publiés par les chercheurs rhodésiens. La dose d'irradiation optimale semble se situer autour de 7000 rad et doit être appliquée à des pupes âgées de plus de 12 jours.

Toutefois, il semble que de meilleurs résultats puissent être obtenus en irradiant des mâles adultes âgés de 24 h à des doses comprises entre 20 000 rad et 25 000 rad.

Ces expérimentations seront reprises avec des effectifs plus importants, à la fois chez G. morsitans morsitans et chez G. tachinoides.

BIBLIOGRAPHIE

AGRICULTURAL RESEARCH COUNCIL OF CENTRAL AFRICA, Annual Report, 1965, p. 63-65.

DAME, D. A., DEAN, G. J. W., FORD, J., "Investigations of the sterile male technique with Glossina morsitans", ISCTR, 10^{e} réunion, Kampala, 24-28 novembre 1964.

POTTS, W. H., Sterilization of tsetse flies (Glossina) by gamma radiation, Ann. trop. Med. Parasit. 52 4 (1958) 484-99.

OOGENESE CHEZ Glossina tachinoides West. ELEVEE AU LABORATOIRE

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Abstract — Résumé

OÖGENESIS IN Glossina tachinoides West. REARED IN THE LABORATORY. A study of the oögenesis cycle has been carried out on female flies of Glossina tachinoides West., reared in the laboratory at 25°C and 70% relative humidity, and mated when three-days-old. A method and a table for the determination of the physiological age is suggested. With the method, which is described, it is possible to estimate the age of a female fly up to about 80 days.

OOGENESE CHEZ Glossina tachinoides West. ELEVEE AU LABORATOIRE. Une étude du cycle de l'oogénèse a été entreprise chez des femelles de Glossina tachinoides West. élevées au laboratoire à 25°C et 70% d'humidité relative, et fécondées à l'âge de 3 jours. Une méthode et un tableau de détermination de l'âge physiologique sont proposés. La méthode décrite permet d'évaluer l'âge d'une femelle jusqu'au 80e jour environ.

INTRODUCTION

La morphologie générale de l'appareil reproducteur des femelles de glossines est maintenant bien connue (fig. 1) et le cycle de l'oogénèse a parfaitement été établi par Saunders. Les études de Saunders ont porté sur quatre espèces de glossines (G. morsitans, G. fuscipes fuscipes, G. pallidipes et G. brevipalpis). Par la suite, Vattier a étudié l'oogénèse chez G. palpalis palpalis et G. fuscipes quansensis, puis Challier a décrit les résultats des recherches faites sur G. palpalis gambiensis.

Nous avons pu, grâce à un élevage de Glossina tachinoides West. maintenu depuis deux ans à l'IEMVT, étudier le cycle de l'oogénèse chez cette espèce.

MATERIEL ET METHODES

Les femelles mortes depuis moins de deux heures et d'âge connu ont été systématiquement disséquées et l'appareil génital isolé. En notant la présence ou l'absence d'œuf ou de larve dans l'utérus, la position, suivant leur taille respective, des ovarioles dans chaque ovaire et leur numérotation selon la méthode décrite par Challier, la présence, pour chaque ovariole, d'un pédicelle ou d'une relique folliculaire et les mensurations des ovarioles, nous avons pu établir un tableau de diagnose de l'âge physiologique des femelles de G. tachinoides jusqu'à la septième ovulation incluse, soit, approximativement, jusqu'à l'âge de 78 jours à 80 jours.

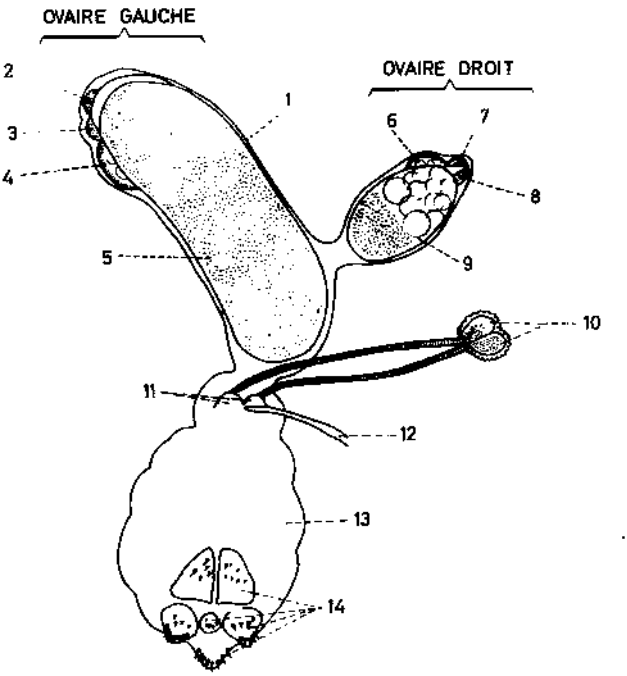

FIG. 1. Organes génitaux d'une femelle âgée de 20 jours.

Abréviations:

1: Gaine de l'ovaire
2: Germarium C
3: Germarium D
4: Follicule D
5: Follicule C (œuf mûr)
6: Follicule A_2
7: Germarium A
8: Germarium B
9: Follicule B
10: Spermathèques
11: Saillies utérines
12: Glande utérine
13: Utérus
14: Plaques génitales

La technique décrite par Saunders permet de classer les femelles de glossines en cinq groupes d'âge, selon que les différents ovarioles ne présentent aucune relique folliculaire (femelles nullipares), ou présentent 1, 2, 3 ou 4 reliques dans l'ensemble des quatre ovarioles. Dans la dernière catégorie sont incluses toutes les femelles ayant effectué plus de quatre ovulations.

Challier a rendu cette méthode plus précise en formulant l'hypothèse selon laquelle les ovulations s'effectuent dans un ordre constant: le premier œuf provient de l'ovariole A, le deuxième de l'ovariole C, le troisième de l'ovariole B, le quatrième de l'ovariole D, le cinquième à nouveau de l'ovariole A, le sixième de l'ovariole C, et ainsi de suite.

En attribuant, dans les ovaires intacts et en position normale, un numéro d'ordre par taille décroissante à chaque ovariole et en groupant ces chiffres selon la position, dans l'espace, des ovarioles, on obtient un nombre repère caractéristique qui, joint à l'étude du nombre de reliques folliculaires, permet de déterminer l'âge d'une femelle jusqu'à

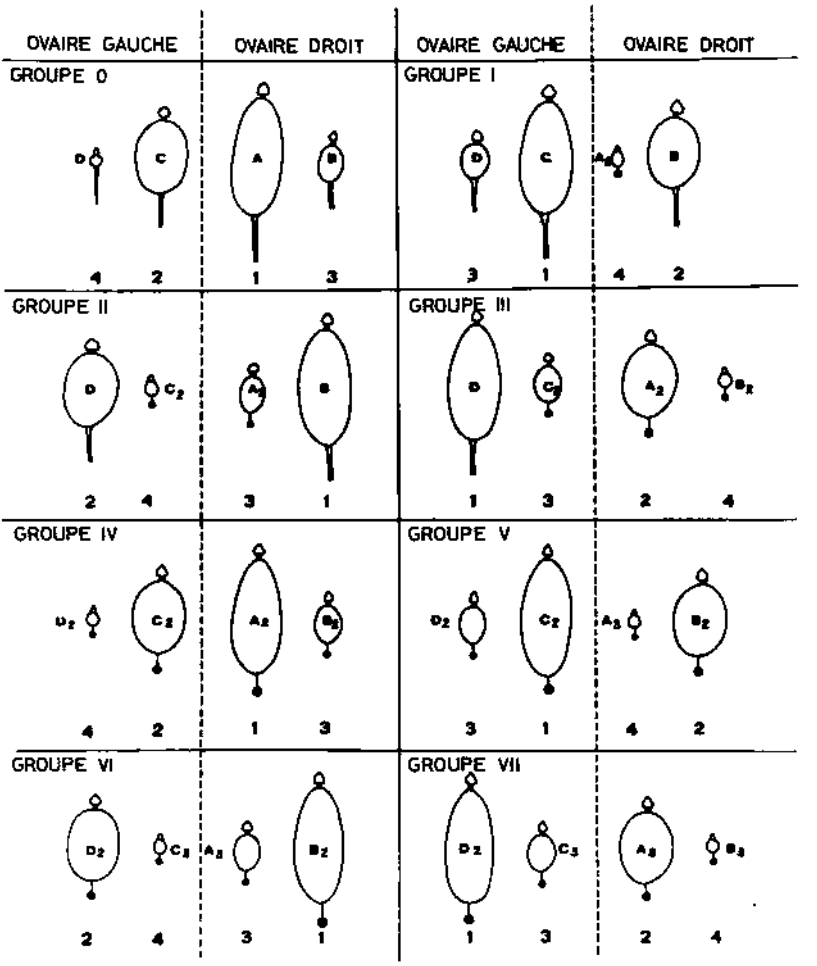

FIG. 2. Tableau de détermination de l'âge physiologique (le reliquat folliculaire est représenté par un point).

la septième ovulation incluse. On peut ainsi distinguer huit groupes d'âge (fig. 2), numérotés de 0 à VII.

Les groupes 0, I, II et III correspondent aux catégories 0, I, II et III de Saunders. Le groupe IV comprend les femelles ayant effectué (4 + 4n) ovulations (4, 8, 12... ovulations). Le groupe V comprend les femelles ayant effectué (5 + 4n) ovulations (5, 9, 13... ovulations). Le groupe VI comprend les femelles ayant effectué (6 + 4n) ovulations (6, 10, 14... ovulations), et le groupe VII les femelles ayant effectué (7 + 4n) ovulations (7, 11, 15... ovulations).

Les seuls nombres repères possibles sont:

4213, qui correspond à des femelles appartenant aux groupes 0 ou IV,

3142, correspondant à des femelles des groupes I ou V,

2431, correspondant à des femelles des groupes II ou VI,

1324, correspondant à des femelles des groupes III ou VII.

Il peut arriver que l'on obtienne un nombre repère théoriquement impossible, tel que 1342. Cette anomalie se produit quelquefois, lorsque l'un des ovarioles contient un œuf mûr. Il est probable que l'œuf mûr, en créant une tension sur la paroi de l'ovaire, a fait glisser le petit ovariole sur sa face concave. Il suffit alors d'intervertir les chiffres correspondant respectivement, dans le même ovaire, à l'œuf mûr et au petit ovariole. Dans l'exemple cité ci-dessus, le nombre repère réel est 3142.

Une imprécision subsiste cependant lorsqu'on ne peut être certain de la présence d'un reliquat folliculaire en dessous d'un œuf mûr dans l'ovariole externe gauche (ovariole D). L'œuf mûr distend en effet considérablement le tube folliculaire, qui devient extrêmement fragile et se déchire lors de la dissection. Dans ces conditions, la dilatation témoin de l'ovulation précédente se confond avec l'intima déchirée et n'est généralement pas repérable. Le groupe d'âge exact reste alors non précisé, la femelle pouvant appartenir aux groupes III, VII, XI, etc.

Une autre imprécision provient de ce que, au-delà du groupe VII, il n'est pas possible de caractériser les femelles ayant eu 8 ovulations ou plus. Toutefois cette imprécision n'affecte que les femelles âgées de plus de 80 jours. Or la répartition des effectifs par groupes d'âge diminue très rapidement à partir du groupe VII. Au laboratoire, la moyenne de vie des femelles de G. tachinoides a été, dans les meilleures conditions, de 50 jours, avec un maximum de 111 jours. Le nombre de femelles vivantes pour 100 femelles écloses se répartit comme le montre le tableau I.

TABLEAU I. REPARTITION PAR GROUPES D'AGE DES FEMELLES VIVANTES POUR 100 FEMELLES ECLOSES

Age (j)	0	1-10	11-20	21-30	31-40	41-50
Groupe d'âge	0	0	I	II	III	IV
Nombre de femelles vivantes	100	71	71	71	61	55
Age (j)	**51-60**	**61-70**	**71-80**	**81-90**	**91-100**	**101-100**
Groupe d'âge	V	VI	VII	VIII	IX	X
Nombre de femelles vivants	42	39	32	3	3	1

RESULTATS ET DISCUSSION

Chez Glossina tachinoides à 25°C et 70% HR, la première ovulation se produit vers le 9e - 10e jour. L'utérus renferme, à cet âge, un œuf produit par l'ovariole A. La larve atteint le stade III en 6 jours environ. La première ponte a lieu entre le 17e et le 20e jour, parfois un peu plus tard. L'œuf suivant, produit par l'ovariole C, se trouve dans l'utérus vers le 20e - 22e jour. Le follicule A_2 présente à ce moment un reliquat folliculaire, témoin de la première ovulation. La deuxième larve est pondue vers le 26e - 30e jour. Les pontes successives ont lieu, après la première, tous les 8-10 jours en moyenne. L'œuf provenant de l'ovariole B est ovulé vers le 30e - 32e jour, et l'œuf provenant de l'ovariole D, vers le 40e - 42e jour. Le deuxième œuf produit par l'ovariole A (follicule A_2) sera ovulé vers le 50e - 52e jour. Dans la plupart des cas les œufs ne succèdent pas immédiatement, dans l'utérus, à la ponte de la larve précédente. Il s'écoule généralement un intervalle d'un à deux jours entre la ponte d'une larve au dernier stade et l'ovulation suivante. Il est donc fréquent, chez une femelle ayant, d'après le nombre de reliquats folliculaires présents dans les ovarioles, produit une ou plusieurs pupes, de trouver un utérus vide.

Chez la femelle nouvellement éclose ou âgée de 1 jour, le follicule A mesure 0,455 mm et le follicule C 0,160 mm. Le follicule B n'est pas encore détaché du germarium. Saunders indique, pour G. morsitans âgée de 0 à 1 jour, les dimensions suivantes: follicule A: 0,486 mm; follicule C: 0,226 mm. Les dimensions du follicule A sont voisines dans les deux espèces; par contre, le follicule C est nettement plus petit chez G. tachinoides. Ces différences s'accentuent avec l'âge, puisque chez G. tachinoides âgée de 8 jours nous avons relevé les dimensions suivantes: A = 1,375 mm; B = 0,150 mm; C = 0,388 mm; D = 0,113 mm. Chez G. morsitans âgée de 8 jours, Saunders indique 1,600 mm pour A; 0,200 mm pour B; 0,506 mm pour C et 0,165 mm pour D. A l'âge de 20 jours, les dimensions sont, respectivement chez G. tachinoides et G. morsitans: A = 0,113 mm et 0,153 mm; B = 0,438 mm et 0,463 mm; C = 1,500 mm et 1,600 mm; D = 0,187 mm et 0,250 mm.

BIBLIOGRAPHIE

BUXTON, P. A., The Natural History of Tsetse Flies, Lewis & Co., London (1955).

BUXTON, P. A., LEWIS, D. J., Climate and tsetse flies: Laboratory studies upon Glossina submorsitans and tachinoides, Phil. Trans. R. Soc. B 224 (1934) 175-240.

CHALLIER, A., Observations sur l'ovulation chez Glossina palpalis gambiensis Vand., 1949, Bull. Soc. Path. exot. 57 5 (1964) 985-91.

CHALLIER, A., Amélioration de la méthode de détermination de l'âge physiologique des glossines, Etudes faites sur Glossina palpalis gambiensis Vand., 1949, Bull. Soc. Path. exot. 58 2 (1965) 250-59.

DETINOVA, T. S., Méthodes à appliquer pour classer par groupes d'âge les diptères présentant une importance médicale, notamment certains vecteurs du paludisme, OMS, Série Monographies n° 47 (1963) 220.

SAUNDERS, D. S., Ovaries of Glossina morsitans, Nature, London 185 (1960) 121-22.

SAUNDERS, D. S., Determination of physiological age for female Glossina morsitans, Nature, London 186 (1960) 651.

SAUNDERS, D.S., The ovulation cycle in Glossina morsitans West. (Diptera-Muscidae) and a possible method of age determination for female tsetse flies by the examination of their ovaries, Trans. R. ent. Soc. London 112 9 (1960) 221-38.

SAUNDERS, D.S., Studies on ovarian development in tsetse flies (Glossina Diptera), Parasitology 51 (1961) 545-64.

SAUNDERS, D.S., Age determination for female tsetse flies and the age composition of samples Glossina palliidipes Aust., Gl. palpalis fuscipes Newst. and Gl. brevipalpis Newst., Bull. ent. Res. 53 (1962) 579-95.

VATTIER, G., Etude de caractères morphologiques et anatomiques en relation avec l'âge physiologique des femelles de glossines, Cahiers ORSTOM, Ent. Méd. 2 (1964) 21-53.

RESULTATS RECENTS D'UN ELEVAGE DE Glossina tachinoides West. ENTREPRIS A MAISONS-ALFORT

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Abstract — Résumé

RECENT RESULTS IN THE BREEDING OF Glossina tachinoides West. AT MAISONS-ALFORT. An artificial breeding of Glossina tachinoides West. carried out at Maisons-Alfort (France) in April 1965, is being continued. The 11th generation has just been reached. This species seems more difficult to breed than G. morsitans. However, the method now being used seems to be producing satisfactory results.

RESULTATS RECENTS D'UN ELEVAGE DE Glossina tachinoides West. ENTREPRIS A MAISONS-ALFORT. Un élevage de Glossina tachinoides West., entrepris à Maisons-Alfort (France) en avril 1965, se poursuit encore à l'heure actuelle. La onzième génération vient d'être atteinte. Cette espèce semble plus délicate à élever que G. morsitans. Cependant, les techniques mises actuellement en œuvre semblent donner des résultats satisfaisants.

INTRODUCTION

Un élevage de Glossina tachinoides West., à partir de pupes (fig. 1) importées de la région de Fort-Lamy (Tchad), a été entrepris au laboratoire d'entomologie de l'IEMVT, en avril 1965. Les premiers résultats obtenus ont fait l'objet d'une communication au début de l'année 1966. Depuis cette époque, nous avons expérimenté différentes techniques d'élevage, afin d'obtenir de meilleurs rendements.

MATERIEL ET METHODES

Rappelons que, au cours de l'année 1965, nos glossines étaient nourries uniquement sur cobaye, que les femelles fécondées étaient maintenues à 25°C et 65,70% d'humidité relative, dans des cages de type Roubaud, à raison de 20 femelles par cage. Les cages étaient recouvertes d'un tulle dont les mailles n'atteignaient pas 1 mm de diamètre. Les femelles déposaient leurs larves dans la cage, mais quelques larves parvenaient à passer à travers les mailles du tulle, et un assez grand nombre d'entre elles restaient coincées dans la maille. Les pupes et les mouches âgées de 1 à 10 jours étaient maintenues dans une autre salle, à 25°C et 80,85% d'humidité relative. L'accouplement a toujours été effectué à l'âge de 3 jours pour les femelles et de 7 jours pour les mâles. Si les femelles de première génération ont fourni, en moyenne, 5,9 pupes par femelle et si leur longévité moyenne a été d'environ 50 jours, dès la deuxième génération le nombre de pupes par femelle est tombé à 2,6 et la longévité moyenne à 30 jours.

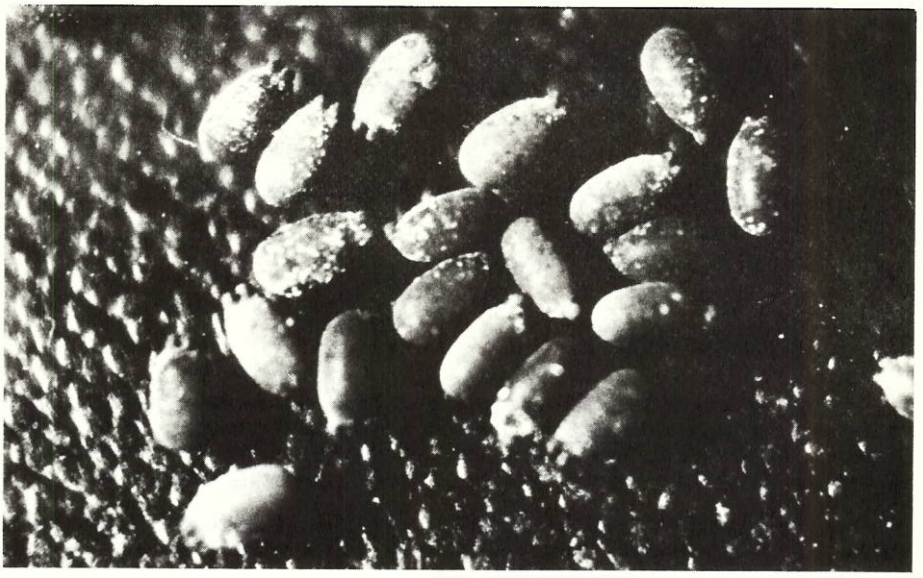

FIG. 1. Pupes de G. tachinoides importées de Fort-Lamy.

Les pourcentages d'éclosion ont été, respectivement, de 87,6 et 79,3. La plupart des pupes non écloses étaient des pupes « étranglées ».

Nous avons pensé que l'alimentation sur cobaye ne convenait peut-être pas et avons nourri, de mars à octobre 1966, nos G. tachinoides sur poule.

Le nombre de pupes par femelle et par mois, qui était de 1,89 en janvier 1966 et 1,24 en février 1966, est passé à 3,54 en mars, pour retomber à 2,01 en avril, 1,81 en mai, 1,48 en juin, 1,86 en juillet, 1,59 en août, 1,64 en septembre et 1,20 en octobre.

Au cours du mois de septembre, la salle d'élevage ayant été repeinte, les mouches ont été placées dans une autre salle où la température n'atteignait que 21 à 22°C et l'humidité 80%. Bien que la salle d'élevage ait été largement aérée pendant 4 jours une fois les travaux de peinture terminés, les G. tachinoides ont été intoxiquées par les émanations de peinture et il s'en est ensuivi une forte mortalité, qui a atteint 2,6% par jour. Ces faits expliquent en partie le faible nombre de pupes par femelle obtenu au cours du mois d'octobre.

RESULTATS

A partir du 3 novembre 1966, les G. tachinoides ont été nourries sur oreilles de lapin, suivant la technique préconisée par le Dr. Nash (fig. 2). En outre, et cela depuis le mois d'août 1966, les femelles fécondées ont été placées dans des cages recouvertes d'un tulle dont les mailles mesuraient 1,5 × 1,5 mm, à raison de 10 femelles par cage.

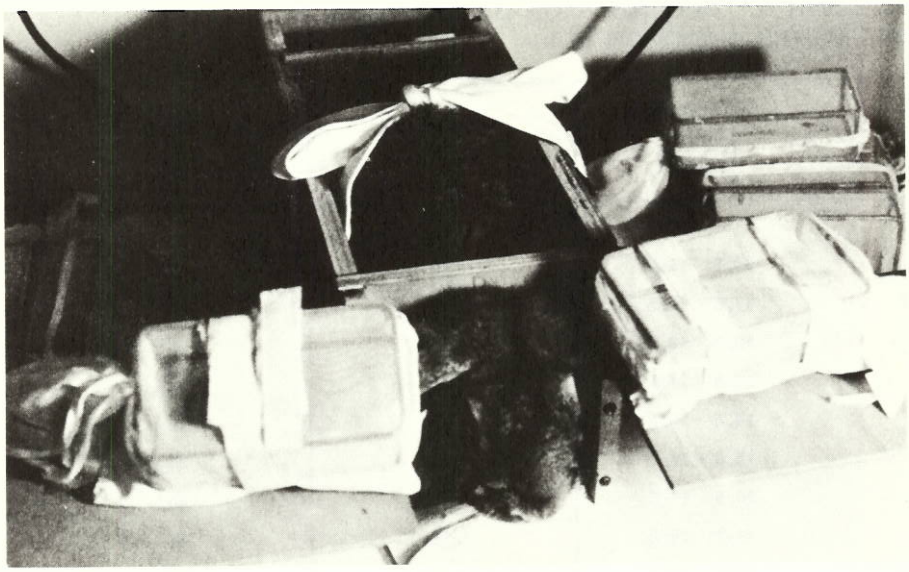

FIG. 2. Nourriture sur lapin à oreilles pendantes.

Depuis que nous avons adopté ce tulle, toutes les larves passent au travers des mailles et pupent au fond du bocal contenant les cages. Nous n'avons ainsi plus aucune pupe étranglée, et les pourcentages d'éclosion atteignent 90%.

A partir du moment où les G. tachinoides ont été nourries sur oreilles de lapin, le nombre de pupes par femelle et par mois a atteint 1,79 en novembre et 2,33 en décembre 1966.

Au bout de 60 jours, le nombre de femelles vivantes était de:

42% (1ère génération)	10% (8e génération)
3% (2e génération)	24% (9e génération)
10% (3e génération)	30% (10e génération).

Le moment où les mouches ont été nourries sur lapin à oreilles pendantes correspond à la fin de la neuvième génération et au début de la dixième génération.

Il semble donc bien que le fait de nourrir les glossines sur lapin ait eu une influence favorable sur la longévité des femelles et sur le nombre de pupes produites.

A partir du 15 novembre 1966, grâce à l'acquisition d'une balance de précision sensible au centième de mg, nous avons pu effectuer des pesées de pupes âgées de 24 h. Pour G. tachinoides, sur 171 pesées effectuées, on obtient la distribution de fréquence figurant dans le tableau I.

Le poids moyen des pupes de G. tachinoides âgées de 24 h est donc de 16,77 mg, avec un écart type de 2,057, soit un poids moyen de 16,77 ± 0,28 mg.

Rappelons que Buxton et Lewis trouvent un poids moyen, pour des pupes de 24 h de G. tachinoides élevées à 24°C, de 14,0083 mg avec une erreur standard de ± 0,671.

TABLEAU I. DISTRIBUTION DE FREQUENCE DES PUPES AGEES DE 24 h

Poids (mg) Limites de classes	Point médian des classes	Fréquence
10,5 - 11,5	11	1
11,5 - 12,5	12	3
12,5 - 13,5	13	5
13,5 - 14,5	14	18
14,5 - 15,5	15	16
15,5 - 16,5	16	27
16,5 - 17,5	17	40
17,5 - 18,5	18	31
18,5 - 19,5	19	13
19,5 - 20,5	20	12
20,5 - 21,5	21	3
21,5 - 22,5	22	2

BIBLIOGRAPHIE

BUXTON, P. A., LEWIS, D. J., Climate and tsetse flies: Laboratory studies upon Gl. submontitans and tachinoides, Phil. Trans. R. Soc. B 224 (1934) 175-240.

ITARD, J., MAILLOT, L., Notes sur un élevage de glossines (Diptera-Muscidae) entrepris, à partir de pupes expédiées d'Afrique, à Maisons-Alfort (France), Revue Elev. Méd. vét. Pays trop. 19 1 (1966) 29-44.

NASH, T. A. M., JORDAN, A. M., BOYLE, J. A., A promising method for rearing Glossina austeni (Newst.) on a small scale, based on the use of rabbits ears for feeding, Trans. R. Soc. trop. Med. Hyg. 60 2 (1966) 183-88.

PHYSIOLOGY OF MYIASIS FLIES INCLUDING Hypoderma bovis

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Abstract

PHYSIOLOGY OF MYIASIS FLIES INCLUDING Hypoderma bovis. Studies in the comparative physiology of myiasis flies were undertaken to facilitate artificial rearing. Radioisotopes were used in biochemical studies.

In central Europe, particularly certain areas of the alpine regions of Bavaria, Austria and Switzerland, and also in the north-west of the Federal Republic of Germany, the ox warble fly is a pest. It can be considered the most important insect pest of cattle in the temperate climatic regions of the northern hemisphere. Excellent systemic insecticides have been developed, for example, the Bayer compound "Neguvon", but since there are, in central Europe in particular, many small private cattle owners, it is extremely difficult to ensure that an insecticide is applied to each animal. Another difficulty in applying the insecticide is that the distance between the producer and consumer of meat, milk, or milk products may be relatively short. For example, many people obtain fresh milk directly from farms, and therefore the insecticide residue problem may be a very serious one in the densely populated regions of central Europe. Eradication of the one-host-parasite Hypoderma by means of the sterile-male technique would therefore be very beneficial.

The chief problem seems to be the need to develop a method of artificially rearing Hypoderma in in-vitro cultures. There have been some unsuccessful attempts. Our research concerns in particular the physiology of the nutrition of the warble fly larvae. It is extremely difficult to obtain material all the year round; only during certain periods of the year are large numbers of flies obtainable from slaughterhouses.

The larvae of the ox warble fly are obligate endoparasites with a highly specialized metabolism. Presumably this is the final stage in a phylogenetical pattern leading to parasitological specialization. To obtain more information and experimental experience, we are comparing physiologically these larvae with those of the facultative myiasis-producing flies such as the blowfly, Calliphora erythrocephala, and semi-obligate myiasis flies such as the screw-worm, Callitroga hominivorax. Calliphora larvae normally live on dead meat, but can enter wounds; screw-worm larvae live normally on and in wounds and penetrate into the host's tissue. They can be reared on meat diets that retain some microbiological organisms. Both seem to be able to serve as "physiological links" to obtain more information about the physiology and nutrition of the warble fly larvae. We measured oxygen consumption and respiratory quotients of Calliphora and Callitroga larvae, followed by wandering larvae of Hypoderma. It is

not known whether the wandering larvae are capable of living under conditions of anoxia or not; the last larval stage in Hypoderma has a connection with the atmosphere. Biochemical methods, working with radioactive labelled substances, etc., are used to determine which nutritional substances are ingested, which are essential, and the role of extra intestinal digestion. The spectrum of enzymes in salivary glands and in different parts of the alimentary tract and a chemical analysis of the semifluid content of the final stage of the Hypoderma larvae are investigated. Histological and histochemical studies on the development of the ovaries in female larvae and pupae of Hypoderma bovis were compared with those of Calliphora (G. Nogge, unpublished).

PHYSIOLOGICAL STUDIES ON THE TSETSE FLY, Glossina morsitans, IN RELATION TO THE PROBLEM OF LABORATORY MAINTENANCE

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Abstract

PHYSIOLOGICAL STUDIES ON THE TSETSE FLY, Glossina morsitans, IN RELATION TO THE PROBLEM OF LABORATORY MAINTENANCE. Field-caught tsetse flies digest their blood meals more rapidly than laboratory-reared flies but they lose this greater digestive capability throughout succeeding hunger cycles if maintained under laboratory conditions. A hypothesis is put forward that protein digestion in the tsetse fly is controlled by the activity of the neuroendocrine system, and evidence is presented that neuroendocrine activity is impaired in the laboratory. It is considered that this impairment is due to lack of environmental stimuli, and results in the production of smaller than normal pupae in the laboratory even though conditions appear to be more favourable than in the field.

The greatest handicap to the development of adequate control measures and to the advancement of our knowledge of the biology of the tsetse fly has been the inability to breed flies in captivity in sufficiently large numbers to provide material for experimental work. With the advent of the sterile-male technique as a control measure for insect pests and the possibility of its application to tsetse, the need to breed flies in large numbers has become even more acute.

The greatest success in rearing a self-supporting colony of tsetse flies was probably achieved by Geigy [1] whose classical experiments with Glossina palpalis are well known. Further notable successes using this species have been achieved in west Africa, but there seems to have been little success elsewhere with other species until very recently.

Azevedo and Pinhão [2] have succeeded in maintaining and increasing the numbers of G. morsitans in their colony in Lisbon and Nash at Bristol is succeeding well with G. austeni [3, 4]. However, the reasons for these successes have yet to be evaluated.

Having spent three years working on the neuroendocrine control of growth and moulting in locusts, I was struck by the idea that the failure of laboratory colonies of tsetse flies to be self-supporting might be attributable to impaired function of the neuroendocrine system under laboratory conditions.

Studies began with the investigation of the anatomy and histology of the tsetse neuroendocrine system. This was a necessary preliminary to experimental work since no previous information was available. In the course of this investigation, no histological evidence of a cycle of secretory activity within the neuroendocrine system was obtained such as exists in other insects. This was not surprising in view of the amount of evidence relating neuroendocrine function to general protein metabolism in insects

and more specifically to sexual maturation [5] and protein digestion [6]. Tsetse flies feed on vertebrate blood and consequently obtain virtually all their nutritional requirements from protein. The adult female fly ovulates continuously whether she has been fertilized or not. Thus there is no stage in the life of the adult fly at which protein metabolism is minimal as there is in many oviparous and omnivorous insects when they have completed the development of their eggs or when they are fed on protein-free diets.

A pair of sensory nerve endings was found on the distensible crop duct at the point where it enters the crop. These resemble "stretch receptors" [7], and are in contact with the neuroendocrine system via the stomatogastric nervous system. Thus a mechanism exists whereby the activity of the neuroendocrine system could be controlled by the degree of distension of the crop with the blood meal [8]. Such a mechanism has already been reported for Locusta migratoria [9].

Thomsen and Møller [6] demonstrated that the presence of the neurosecretory cells in the brain of the blowfly, Calliphora erythrocephala, was essential for the production of intestinal proteolytic enzymes (proteases). Secretion of proteases in the tsetse fly is restricted almost entirely to the middle segment of the midgut [10] and fluid is extracted from the meal in the anterior segment. Lester and Lloyd [11] showed that almost two thirds of the weight of the original meal is lost in this way during the 24 hours following its ingestion. Bursell [12] has shown that the amount of water extracted from the meal is proportionately less in small meals than in large meals. Thus the quantity of food passing through the digestive portion of the midgut at any particular time bears little relation to the size of the meal originally ingested.

Consequently, if a similar relationship existed between the median neurosecretory cells of the tsetse fly brain and the secretion of proteases in the midgut, and the stretch receptor hypothesis was correct, the amount of hormone released to produce enzyme in the midgut would be proportional to the degree of distension of the crop and thus to the size of the meal ingested. There should be a straight line relationship between enzyme activity and meal size. This was indeed the case, the linear relationship being established 24 hours after the ingestion of the meal and persisting throughout the hunger cycle [13].

When the crop was distended with saline alone, no increase in protease activity was recorded in the midgut. However, provided at least 10% of the ingested fluid was blood, the development of protease activity was proportional to the degree of distension of the crop and not to the amount of blood in the meal. Pursuing this line further, it was found that the presence of blood serum and not the corpuscles was essential for the appearance of active enzyme in the midgut [13]. Thus it has been suggested that the size of the meal ingested controlled the liberation of hormones into the haemolymph by causing impulses to pass from the stretch receptors on the crop duct along the oesophageal nerves to the neuroendocrine system. The hormone or hormones stimulate the production of an enzyme precursor in the middle segment of the midgut, which is then activated in the presence of blood serum.

Following up the idea that failure of self-supporting laboratory colonies could be attributed to an impairment of neuroendocrine function, a comparison was made between the rates of digestion in field flies and

laboratory-reared flies maintained under identical environmental conditions. Measurements were made of midgut protease activity and the results checked by measuring rates of excretion. Field flies digested their blood meals more rapidly and had a higher protease activity than flies hatched and reared in the laboratory [14].

Since the field flies fed from a bait ox in their natural environment, whereas the laboratory-reared flies were fed on guinea pigs, the possibility that the blood of the different hosts was causing the variation in the rate of digestion had to be considered. However, further experiments in which laboratory-hatched flies were fed on a variety of vertebrate hosts, including cattle, showed that the type of host had no effect on the rate of digestion of the meal taken [15]. Furthermore, when field flies were maintained in the laboratory on guinea pigs, their rate of digestion decreased over three hunger cycles almost to the same level as that of laboratory-hatched and -reared flies [16].

Therefore there was now ample circumstantial evidence for a neuroendocrine control of digestion in tsetse flies and that the activity of the neuroendocrine system was impaired under laboratory conditions.

Since the source of the blood meal did not affect the impaired rate of digestion of the flies fed in the laboratory, the choice of a laboratory host animal was of secondary importance in the successful maintenance of self-supporting colonies of tsetse flies.

The environment plays an important part in influencing neuroendocrine function in other insects [17]. Some form of environmental preconditioning effect may therefore be essential to the tsetse fly. In this context, the greater activity of field flies imposed by the necessity of seeking a suitable host, and in the male the swarming behaviour round moving objects, referred to by Bursell [18] as the "sexual appetitive" phase, might be important. Restricted activity in laboratory-reared flies may account for the failure of such colonies to be self-supporting in view of their poorer nutritional state.

A study of the recently emerged, teneral fly provided more evidence in favour of a neuroendocrine control of proteolytic enzyme secretion [19]. It was found that upon emergence, the teneral fly had low intestinal protease activity but that activity increased threefold during the 24 hours following emergence.

The behaviour of the fly upon emergence fitted in well with the stretch receptor hypothesis. The fly achieves its full size by inflating its crop with air which could activate the proposed neurohormonal pathway in the same way as ingestion of a blood meal. The middle segment of the midgut contains the remnants of the last larval meal of "uterine milk", which could satisfy the requirements for the appearance of active enzyme on the basis of the blood serum experiments. An analysis of the components of the uterine milk both in field- and laboratory-reared flies, for comparison with the composition of vertebrate blood serum, would be valuable.

By puncturing the ptilinum of a newly emerged fly, the fly was unable to expand and the crop did not inflate. Such flies failed to increase their midgut protease activity above the level of the newly emerged fly. These flies lived for up to 72 hours, ran about actively and even attempted to probe, although none was successful at obtaining a meal.

As a control experiment, the ptilinum of flies that had recently expanded but had not yet hardened was punctured. These flies remained expanded and their midgut protease activity rose to the level of normal flies within 48 hours. Flies punctured before expansion and then injected with extracts of brain tissue from normal flies showed a slight but significant increase in protease activity over similar flies injected with an extract of thoracic muscle, which showed no increase.

There is now conclusive evidence therefore that the increase of midgut protease activity in tsetse flies is associated with the distension of the crop. The presence of blood serum or of uterine milk in the midgut is also necessary, and it seems that the brain of the fly is also involved.

The individual attention given to tsetse flies in captivity, the removal of the numerous hazards attendant upon feeding in the natural environment and the provision of a regular food supply in the laboratory, might be expected to result in the production of pupae larger than those found in the field. Such is not the case, which indicates that the metabolism of laboratory-reared flies is impaired. Until a method is found to demonstrate the activity of the neuroendocrine system in the tsetse fly, this metabolic impairment in terms of a hormonal irregularity cannot be explained completely.

REFERENCES

[1] GEIGY, R., Elevage de Glossina palpalis, Acta trop. 5 (1948) 201.

[2] AZEVEDO, J.F., PINHÃO, R.da C., "The maintenance in the laboratory of a colony of Glossina morsitans (Diptera) since 1959", World Health Publication, WHO (Vector Control) 94.

[3] NASH, T.A.M., JORDAN, A.M., BOYLE, J.A., A promising method for rearing Glossina austeni (Newst.) on a small scale, based on the use of rabbits' ears for feeding. Trans. R. Soc. trop. Med. Hyg. 60 2 (1966) 183.

[4] NASH, T.A.M., KERNAGHAN, R.J., BOYLE, J.A., The large-scale rearing of Glossina austeni (Newst.) in the laboratory: 1. The use of pregnant and non-pregnant goats as hosts, Ann. trop. Med. Parasit. 60 (1966) 39.

[5] HIGHNAM, K.C., The histology of the neuroendocrine system of the adult female desert locust Schistocerca gregaria, Q. Jl microsc. Sci. 102 (1961) 27.

[6] THOMSEN, E., MØLLER, I.B., Influence of neurosecretory cells and of corpus allatum on the intestinal protease activity in the adult Calliphora erythrocephala Meig., J. exp. Biol. 40 (1963) 301.

[7] FINLAYSON, L.H., LOWENSTEIN, O., A proprioceptor in the body musculature of Lepidoptera, Nature, Lond. 176 (1955) 1031.

[8] LANGLEY, P.A., The neuroendocrine system and stomatogastric nervous system of the adult tsetse fly Glossina morsitans, Proc. zool. Soc. Lond. 144 (1965) 415.

[9] CLARKE, K.U., LANGLEY, P.A., Studies on the initiation of growth and moulting in Locusta migratoria migratorioides R. and F.: IV. The relationship between the stomatogastric nervous system and neurosecretion, J. Insect Physiol. 9 (1963) 423.

[10] WIGGLESWORTH, V.B., Digestion in the tsetse fly, Parasitology 21 (1929) 288.

[11] LESTER, H.M.O., LLOYD, L., Notes on the process of digestion in tsetse flies, Bull. ent. Res. 19 (1928) 39.

[12] BURSELL, E., Loss of water by excretion and defaecation in the tsetse fly, J. exp. Biol. 37 (1960) 689.

[13] LANGLEY, P.A., The control of digestion in the tsetse fly Glossina morsitans: enzyme activity in relation to the size and nature of the meal, J. Insect Physiol. 12 (1966) 439.

[14] LANGLEY, P.A., The control of digestion in the tsetse fly Glossina morsitans: a comparison between field flies and flies reared in captivity, J. Insect Physiol. 13 (1967) 411

[15] LANGLEY, P.A., The effect of environment and host type on the rate of digestion in tsetse flies (Glossina morsitans Westwood.), Bull. ent. Res. 57 (1966) 39.

[16] LANGLEY, P.A., Digestion in the tsetse fly Glossina morsitans: the effect of feeding field flies on guinea pigs under laboratory conditions, Bull. ent. Res. 57 (1967) 447.
[17] WIGGLESWORTH, V.B., Advances in Insect Physiology 2, Academic Press, London (1964).
[18] BURSELL, E., The behaviour of tsetse flies (Glossina swynnertoni Austen) in relation to problems of sampling, Proc. Roy. ent. Soc. Lond. A36 (1961) 9.
[19] LANGLEY, P.A., Experimental evidence for a hormonal control of digestion in the tsetse fly, Glossina morsitans: a study of the larva, pupa and teneral adult fly, J. Insect Physiol. 13 (1967) 1921.

SUCCESSES ACHIEVED IN THE LIBERATION OF THE REPRODUCTIVE POTENTIAL OF Glossina austeni

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Abstract

SUCCESSES ACHIEVED IN THE LIBERATION OF THE REPRODUCTIVE POTENTIAL OF Glossina austeni. When rearing larviparous insects that consequently have a low reproductive potential, maximum efficiency for each life cycle stage is essential. Methods of achieving and maintaining this efficiency in the laboratory population of tsetse are described.

BASIC REARING TECHNIQUE

Fertilization

We have raised the insemination rate of tsetse flies, as revealed by dissection after death, from 89 [1] to 97%, leaving little scope for improvement. Briefly, a 3-day-old female that had fed, was placed in a tube for 24 hours with a 15-day-old male that had not mated for 5 days. (Mass mating in a large cage gave a rate of only 89%.)

Adult maintenance conditions

A fly is never touched by hand. On the 4th day of life the "fertilized" females are transferred to cages: either 25 to a large cage suitable for application to the flanks of goats or calves [2], or 10 to a similar but smaller cage suitable for application to the ears of rabbits [3]. In both cases the initial allowance of space per female is 4 in^3 (65.5 cm^3), a figure which our experiments suggest is near the optimum. The type of cage is also a very important factor. Our original container was a box with wooden sides, top and bottom, and netting front and back; our current container is a modified Geigy-type cage, a shallow, rectangular wire frame, covered in black Terylene netting. Five hundred females kept in the new cages yielded 70% more pupae than 500 females kept in the old boxes; further, the mean weight of the pupae was 1.7 mg greater in the new cages [2]. The larvae wriggle through the netting of the cages and pupate in trays.

Since the full reproductive potential of Glossina was approached with these Geigy-type cages when the females fed on rabbit ears, it is doubtful whether the cage factor can be improved; for the same reason the climatic maintenance conditions (about 25°C and 80% relative humidity) are believed

to be near the optimum for G. austeni. A diurnal light rhythm is supplied. For 12 hours of the day the fly-racks receive light intensities ranging from 5.4 to 26.9 lx (0.5 to 2.5 foot candles). All flies were offered food for 15 min daily, Sundays excepted; we found that 7-day feeding improves survival and reproduction but is impractical with a small staff [4].

Pupal maintenance conditions

A pupa is never touched by hand. In our mass-production technique, the pupae are collected from the sand-filled trays at intervals of three weeks, and are then buried in sand in jars having bottoms of open-mesh Terylene voile. The jars are placed on grids just above wet sand, enabling an almost saturated atmosphere to percolate into the pupal environment, without the pupae becoming wet. The emergence rate is 98%, but 5% of the emergences are crippled, in that the wings fail to develop fully; hence the effective emergence rate is only 93%. This is a weakness in our technique; the cause of crippling is being investigated.

Precautions against contamination by insecticide

As we learnt from bitter experience [1], attempts to rear Glossina in Europe are liable to be vitiated by insecticidal contamination, unless it is postulated that minute, immeasurable contamination can lower the survival rate and reduce fecundity, without producing any classical symptoms of toxicity in the fly, and that every animal purchased is liable to be contaminated and therefore should be washed in hot water and soft soap before entering the animal house, as should articles with which tsetse will come in contact. Veterinary surgeons should be given clean coats before examining sick animals. No aerosols, gardening clothes, modern detergents or adhesives should be used in the laboratory. The animals, such as goats, should be close-clipped fortnightly and washed at two-monthly intervals to guard against a build-up of contamination from hay and other sources. Since we followed these precepts, no experiments have been ruined by inexplicably high mortality.

FLY FEEDING AND PERFORMANCE IN RELATION TO THE HOST

Given correct maintenance conditions, the weight of the newly emerged larva will depend upon the amount of blood taken up and successfully metabolized by the mother during the 9-day interlarval period; the amount will depend on the attractiveness of the host and the availability of the blood. Longevity and pupal production are greatly influenced by the host.

Domestic pigs were tested, but proved unattractive to G. austeni and difficult to handle. Sheep were very attractive, but only 2% of the flies succeeded in engorging; possibly English sheep are too fat. Calves, goats and rabbits are the hosts we investigated. To prevent permanent skin damage, hosts are given 72-hours rest between fly feeding [5].

Relatively large animals such as calves and goats are mounted on trolleys and wheeled into the Fly Handling Room, where four cages of flies, each initially holding 25 flies, are strapped on to the flanks [6].

This is undoubtedly the largest-scale successful feeding technique yet devised for tsetse flies, enabling up to 400 flies per hour to feed on each host; further, a profit of 4.5 pupae per female can be achieved when using this method.

A technique was devised for feeding flies on the ears of lop-eared rabbits [3]. The rabbit is placed in a specially designed box incorporating two horizontal platforms on which each ear can rest. A convex pad is placed under the ear, and the cage on top; two elastic straps, attached to the pad, are then stretched over the top of the cage and secured. Each cage initially holds 10 flies, enabling up to 80 flies per hour to feed on each rabbit.

When using calves and goats, owing to the small numbers of tsetse surviving, the experiments are closed on Day 139 after mating; this is the theoretical end of the 14th reproductive cycle. When using rabbits, longevity is so much better that the experiments are continued until the death of the last fly, which may be up to nine months after the experiment began.

Any excess in yield above 2.5 pupae per female is considered profit. (Two pupae must be retained to provide a male and a female for the breeding stock and 0.5 allowed for wastage due to deaths between pupation and fertilization, and for insemination failure.) It follows that when yields are expressed as "pupae per 100 females", 250 pupae must be deducted from the total yield to ascertain the profit.

The results shown in the upper and lower portions of Table I are not comparable, since the former are based on 7-day feeding and the box-type cage now discarded, and the latter on 6-day feeding and the new, improved cage.

TABLE I. YIELDS OBTAINABLE WHEN USING DIFFERENT HOSTS

	Survival by Day 139 (%)	Pupae per 100 females	
		No. of pupae	Profit
7-day feeding. Old cage. To Day 139			
Calf	11	510	260 (412)[a]
Goat (non-pregnant)	2	315	65 (219)[a]
6-day feeding. New cage. To Day 139			
Pregnant goat	14	711	461
Goat (non-pregnant)	4	427	177
6-day feeding. New cage. To death			
Rabbit ears	49	1142	892
Goat (non-pregnant)	12	536	286

[a] Estimated profit had the new type of cage been used.

Calf/non-pregnant goat [4]

The better yield from calves is due to better survival only, but the addition of 7-day feeding further improves survival and also the regularity of larviposition. The pupal weight is not raised. This method is impractical in controlled climate rooms in Europe: the calves smell, defecate unpleasantly, and are difficult for female technicians to handle; Sunday-feeding is unpopular. The method, with a probable profit of 400 pupae per 100 females, is well worth a trial in Africa: the flies could be fed in grass sheds, male labour is available and Sunday-feeding easier to arrange.

Pregnant/non-pregnant goat [2]

The better yield from pregnant goats is due both to better survival and more regular larviposition; the pupal weight was increased by 1 mg to 21.1 mg.

Rabbit ears/non-pregnant goat [7]

The much better yield from rabbits is due to the amazing survival rate and regularity of larviposition; the mean age at death was 139 days with rabbit and 79 days with goat. The profit was almost 9 pupae per female (in a recent experiment it was 10.2). The mean pupal weight at 23.0 mg almost equalled the estimated wild weight on pupation of 23.6 mg [1, 8]. The exceptional goat-fed control yields in this experiment may be due to the use of the small rabbit-type cage for the sake of conformity, and to continuing the experiment until the death of the last fly.

The results may be summarized as follows:

Non-pregnant goats	yield about	400-500	pupae/100 females	– profit 150-250
Pregnant goats	yield about	700	pupae/100 females	– profit about 450
Rabbit ears	yield about	1 140	pupae/100 females	– profit nearly 900

In assessing the profit available when using the different hosts it must be remembered that, allowing 15 min feeding-time per cage, an animal the size of the goat or calf can feed 400 flies per hour as against 80 on the rabbit, a fivefold economy in feeding time. Current work suggests that when using rabbits the feeding time could be reduced to 10 min, raising the figure to 120 females per rabbit per hour. Our male stock is fed entirely on goats; even so, we have far more males than are needed for mating.

SOME IMPLICATIONS FROM THE RABBIT-EAR FINDINGS

With the introduction of the efficient rabbit-ear technique, we can now get a much better idea of the potential rate of increase for Glossina. Estimates, based on the pupal period, yields and mortality rates found at all stages of the life history under our maintenance conditions, may facilitate studies by the experts on the feasibility of rearing tsetse for the control of Glossina by the sterile-male technique.

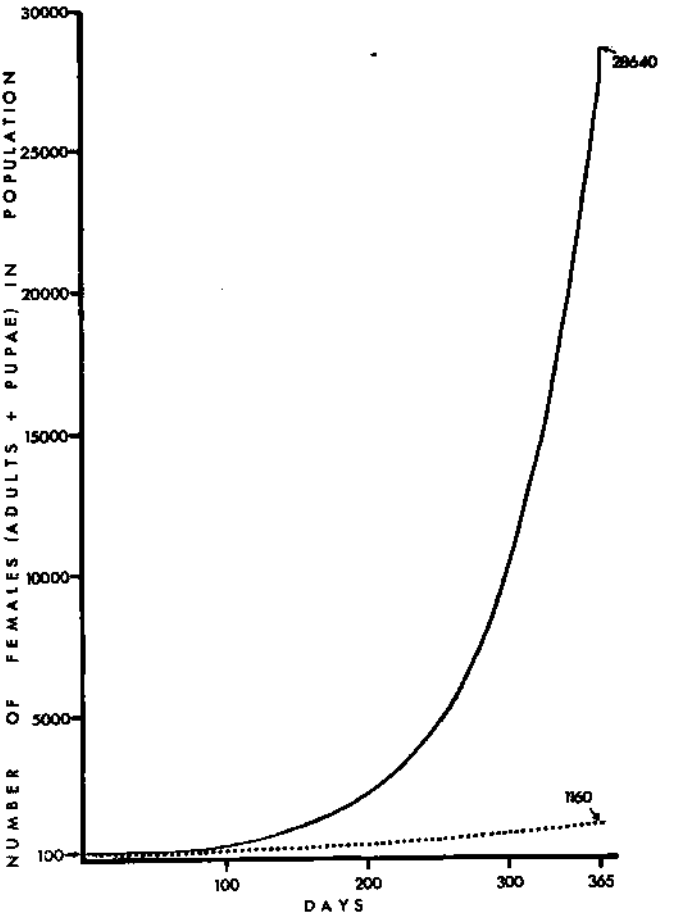

FIG. 1. Estimated rates of increase for a population of Glossina.

..... Glasgow (1963)

—— Rabbit-ear technique (1966).

An estimate was made, and a curve plotted, of the rate of increase for a population of G. austeni fed on rabbit ears when all offspring are added to the breeding stock, for comparison with a similar curve prepared from Glasgow's calculations for Glossina, based on the data available from earlier and less efficient rearing techniques [9] (Fig. 1). Starting with 100 females (adults and pupae) of a stable age distribution, and using our technique, within one year the female population will have risen exponentially to 28 640; whereas Glasgow's calculations indicated an increase from 100 to only 1160 females. Comparison with the rate of increase reported by Azevedo and Pinhão for their Lisbon colony of G. morsitans is difficult because their published data refer only to adult flies [10]; it probably lies between the two curves shown on Fig. 1, as their curve closely follows Buxton's [11] hypothetical calculation based on a mean age of 70 days and a mean fecundity of 6 pupae per female, as against our comparable figures of 139 days and 11.4 pupae.

Now let us consider a colony in which sufficient pupae are retained to maintain a constant size, and all surplus is sterilized and released. The size of the colony will depend upon the number of rabbits that can be kept. If a rabbit is used every third day, it can certainly feed 160 female tsetse without exsanguination. On this basis Dr. C. F. Curtis, who has recently joined us, estimates that a stock of 300 rabbits would maintain a colony of 15 150 adult females and 850 stud males. Such a colony would yield 8290 pupae per week of which 6550 could be sterilized and liberated; ignoring any losses due to sterilization, 6080 of these should emerge per week of which 3040 will be males and 3040 will be females. From the unsterilized pupae all the emerging females and 176 of the males must go into the breeding stock; if desired, the remaining 634 males could be sterilized and released. For any other desired number of releases the numbers in the breeding colony and the number of rabbits can be calculated by simple proportions. Thus the release of 10 000 young adult males per week would necessitate a colony of 41 100 adult females, 2318 stud males and a stock of 814 rabbits.

CURRENT OUTPUT OF THE LABORATORY

We are periodically forced to destroy hundreds of tsetse flies in our self-maintaining colony because the demand for material by other workers is insufficient to keep down the population. We are trying to reduce our stock so as to provide a surplus for distribution equal to a demand for about 700 pupae per month. At present more than 1200 pupae are being deposited weekly of which only one eighth originate from rabbit-fed flies, as the bulk of our research is being undertaken with goats in an effort to produce an even larger-scale rearing technique. We have not received pupae from Zanzibar for many months.

REFERENCES

[1] NASH, T.A.M., JORDAN, A.M., BOYLE, J.A., The large-scale rearing of Glossina austeni (Newst.) in the laboratory: I. The use of pregnant and non-pregnant goats as hosts. Ann. trop. Med. Parasit. 60 (1966) 39.

[2] NASH, T.A.M., JORDAN, A.M., BOYLE, J.A., The large-scale rearing of Glossina austeni (Newst.): III. Confirmation of the value of pregnant goats as hosts. Ann. trop. Med. Parasit. 60 (1966) 469.

[3] NASH, T.A.M., JORDAN, A.M., BOYLE, J.A., A promising method for rearing Glossina austeni (Newst.) on a small scale, based on the use of rabbits' ears for feeding. Trans. R. Soc. trop. Med. Hyg. 60 (1966) 183.

[4] JORDAN, A.M., NASH, T.A.M., BOYLE, J.A., The large-scale rearing of Glossina austeni (Newst.) in the laboratory: II. The use of calves as hosts on seven days of the week. Ann. trop. Med. Parasit. 60 (1966) 461.

[5] NASH, T.A.M., JORDAN, A.M., WRIGHT, A.I., A method for the prevention of skin reactions in goats used for feeding tsetse flies, Glossina spp., Ann trop. Med. Parasit. 59 (1965) 88.

[6] NASH, T.A.M., KERNAGHAN, R.J., The feeding of haematophagous insects on goats and sheep: techniques for host restraint and cage application, Ann. trop. Med. Parasit. 58 (1964) 168.

[7] JORDAN, A.M., NASH, T.A.M., BOYLE, J.A., The rearing of Glossina austeni (Newst.), using lop-eared rabbits as hosts: I. Efficacy of the method (in press).

[8] NASH, T.A.M., KERNAGHAN, R.J., The weight and viability of puparia of Glossina austeni in relation to the conditions provided for pupal development, Ann. trop. Med. Parasit. 59 (1965) 226.

[9] GLASGOW, J.P., The Distribution and Abundance of Tsetse, Pergamon Press, Oxford (1963).

[10] AZEVEDO, J.F. de, PINHÃO, R. da C., The maintenance of a laboratory colony of Glossina morsitans since 1959, Bull. Wld Hlth Org. 31 (1964) 835.

[11] BUXTON, P.A., The Natural History of Tsetse Flies, London School of Hygiene and Tropical Medicine, Memoir No.10, H.K. Lewis & Co., Ltd., London (1955).

REPARTITION GEOGRAPHIQUE ET ECOLOGIE SPECIALE DES GLOSSINES (DIPTERA, MUSCIDAE) AU SENEGAL

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(Présenté par J. Itard)

Abstract — Résumé

GEOGRAPHICAL DISTRIBUTION AND SPECIAL ECOLOGY OF THE GLOSSINA (DIPTERA, MUSCIDAE) IN SENEGAL. The genus Glossina occupies the southern half of the Republic of Senegal and some other small areas along the Atlantic coast. Glossina morsitans submorsitans and Glossina palpalis gambiensis are among the commonest species. A third species (Glossina longipalpis), which is considered to be scarce, has been recorded in some confined areas.

REPARTITION GEOGRAPHIQUE ET ECOLOGIE SPECIALE DES GLOSSINES (DIPTERA, MUSCIDAE) AU SENEGAL. En dehors de quelques îlots résiduels sur la côte atlantique, les glossines n'occupent que la moitié méridionale du Sénégal. Glossina morsitans submorsitans et Glossina palpalis gambiensis y sont les espèces les plus fréquentes. Une troisième espèce, Glossina longipalpis, considérée comme très rare, est signalée dans quelques plages de faible étendue.

INTRODUCTION

Les premières études de répartition géographique des glossines au Sénégal remontent au début du siècle et sont liées à l'épidémiologie de la maladie du sommeil [1, 2]. Au cours d'une mission au Sénégal en 1915, Roubaud délimite les zones à tsétsés de la Petite-Côte et du Bas-Saloum [3]. En 1912, Dufougère publie une étude sur la maladie du sommeil et les trypanosomiases animales en Casamance [4]. En 1916, Bouet parcourt les zones à glossines du Sénégal en longeant le chemin de fer transcontinental [5]. Moulanier et Diouf font, en 1952, le bilan de dix années de lutte contre la maladie du sommeil sur la Petite-Côte du Sénégal et dans la région des Niayes [6]. L'étude de la répartition géographique des glossines dans les Niayes est reprise par Morel en 1964 aux fins de définir des moyens de lutte appropriés [7].

De 1908 à 1961 plusieurs cartes d'épidémiologie des trypanosomiases et de répartition géographique des glossines dans l'Ouest africain traitent du Sénégal; citons: Gouzien [8], Roubaud, Gaschen [9], les Services géographiques de l'AOF [10], Mornet [11], Potts [12], Rickenbach [13].

En 1965 et 1966, des prospections effectuées dans le cadre d'études sur les trypanosomiases animales nous ont permis de préciser la répartition géographique actuelle des glossines.

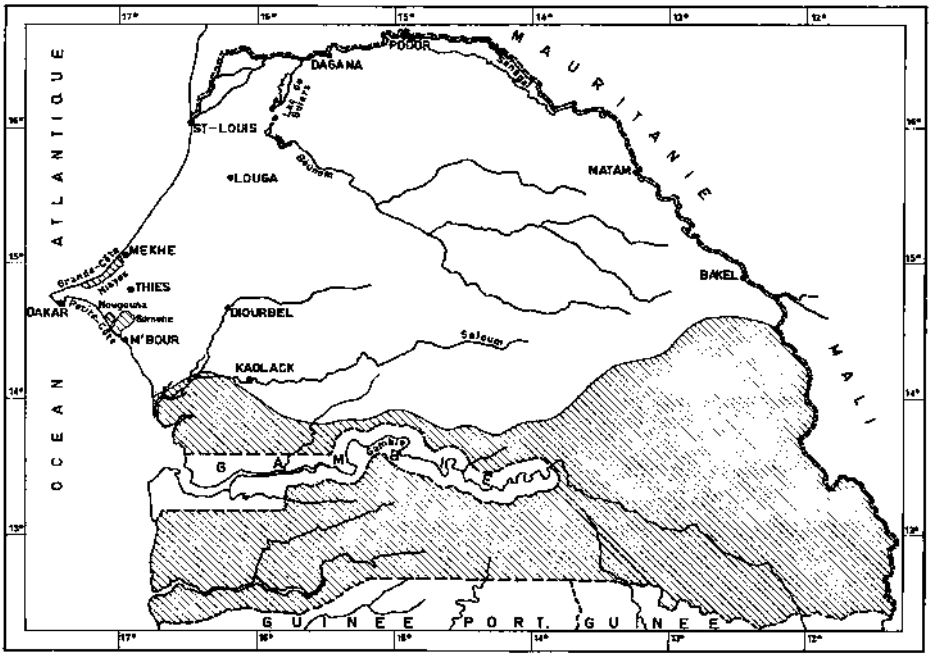

FIG. 1. Répartition des glossines au Sénégal (surfaces hachurées).

REPARTITION GEOGRAPHIQUE

Mis à part quelques îlots résiduels sur la côte atlantique, les glossines n'occupent que la moitié méridionale du Sénégal. Leur limite nord de distribution est comprise entre le 14e et le 15e parallèles (fig. 1).

Trois espèces sont mentionnées au Sénégal:

Sous-genre Nemorhina:

a) Glossina palpalis gambiensis, Vanderplank 1949.

Sous-genre Glossina:

b) Glossina morsitans submorsitans, Newstead 1910.

c) Glossina longipalpis, Wiedmann 1830.

Glossina palpalis gambiensis est rencontrée dans des plages de végétation particulière le long de la Grande-Côte et de la Petite-Côte atlantiques: Niayes, Somone, Nougouna. Plus au sud, l'espèce est abondante dans l'embouchure du Saloum et en Casamance. Sa présence en ces régions est favorisée par un très haut degré d'humidité: mangrove côtière et forêts denses. A l'est, elle est liée à l'existence de cours d'eau: réseau fluvial de la Gambie et de la Casamance.

Glossina morsitans submorsitans est, de loin, la plus fréquente. Elle occupe l'ensemble des forêts, d'ouest en est.

Glossina longipalpis, mentionnée plusieurs fois en Casamance, n'a cependant été décelée dans aucune de nos prospections malgré les étapes qui lui ont été consacrées en particulier. Ne pouvant point contester l'existence de l'espèce, force est de reconnaître son extrême rareté. Il est à noter que G. longipalpis ne figure pas dans les collections que nous avons pu consulter: collections établies par P. C. Morel depuis 1956 au Laboratoire national de l'élevage et de recherches vétérinaires, Dakar; collections de l'Institut français d'Afrique noire à Dakar. Les limites assignées à son aire d'extension sont celles données par Potts dans sa carte synthétique de 1953 [12].

ECOLOGIE SPECIALE

Glossina palpalis gambiensis est liée, au Sénégal, à quatre types d'habitat:

a) La forêt dense humide

Elle couvre la partie occidentale de la Casamance, où les précipitations annuelles varient entre 1000 et 1500 mm de pluies. De plus, la présence de nombreux marigots côtiers entretient toute l'année une humidité relative très élevée, favorable au maintien de l'espèce sans grande fluctuation saisonnière.

b) La mangrove

Les forêts de Rhizophora sont étroitement liées au réseau hydrographique. Quelques forêts de palétuviers se trouvent entre Sokone et la frontière gambienne, à l'embouchure du Saloum. Mais c'est en Casamance qu'elles trouvent leur plus grande étendue, notamment en bordure des rivières continentales, en eau douce, où la mangrove est souvent dense et très élevée. Glossina palpalis gambiensis s'y maintient toute l'année.

c) Les palmeraies

Les peuplements de palmiers à huile (Elæis guineensis) sont surtout localisés autour des bassins d'inondation de rivières, transformés en marécages pendant les crues d'hivernage; il en est ainsi de la Casamance. Sur la Grande-Côte atlantique des palmeraies poussent en îlots séparés dans des bas-fonds argileux d'un type particulier (Niayes). G. palpalis gambiensis colonise ces palmeraies. Sa population varie et a tendance à se confiner autour des collections d'eau à mesure que s'accentue la sécheresse.

d) La végétation riveraine

L'espèce occupe les berges de la plupart des rivières de Casamance et des affluents de la Gambie dans le Sénégal oriental. Sur la Petite-Côte atlantique, deux petits bras de mer (Nougouna et Somone) sont bordés d'une végétation infestée de glossines.

Glossina morsitans submorsitans est associée aux forêts claires et à la savane arborée. Il n'y a pas de grandes particularités écologiques à signaler dans les régions qu'elle occupe au Sénégal. Elle remonte au nord un peu au-delà du 14e parallèle mais s'arrête quand apparaissent les savanes arbustives et clairsemées à Acacia.

CONCLUSION

La répartition géographique actuelle des glossines n'est pas en tous points conforme à celle qui était admise il y a quelque trente années.

Glossina palpalis gambiensis était signalée le long de la côte occidentale depuis le sud de Saint-Louis jusqu'à la frontière gambienne. De nos jours, l'espèce s'arrête au 15e parallèle (Mékhé). Sa disparition au nord est due au déboisement. Au sud, l'espèce s'étendait de M'Bour à Fatick. Elle a disparu de cette partie du territoire. De même on ne la trouve plus dans le Panntior, au nord de la Nougouna, la rivière qui s'y trouvait ayant été asséchée et sa végétation coupée.

Glossina morsitans submorsitans a, dans le Sénégal oriental, une plus grande étendue qu'il n'était admis. On la trouve en suivant la piste de Goudiry à Bakel, jusqu'à une vingtaine de kilomètres avant cette dernière ville.

Glossina longipalpis était mentionnée dans les Niayes dans les mêmes localités que celles qui hébergent G. palpalis. Il est avéré qu'il s'agissait d'erreurs dans la détermination, Glossina palpalis étant en fait la seule espèce qui s'y trouve.

Dans toutes les régions infestées de glossines, les animaux domestiques contractent la trypanosomiase. Trois principales espèces pathogènes sont transmises par les glossines au Sénégal: Trypanosoma vivax, T. congolense et T. brucei. Toutefois l'aire d'extension de T. vivax déborde nettement celle des glossines et recouvre pratiquement l'ensemble du pays, du fait qu'au nord de la limite de distribution des tsétsés les trypanosomiases sont transmises mécaniquement par d'autres Diptères.

REFERENCES

[1] THIROUX, A., Les villages de ségrégation et de traitement de la maladie du sommeil, Fonctionnement d'un de ces villages à Saint-Louis du Sénégal, J. off. Afr. occid. fr. 7 (suppl.) (1909) 8 p.

[2] THIROUX, A., ANFREVILLE DE LA SALLE, L.de, La maladie du sommeil et les trypanosomiases animales au Sénégal, Baillières, Paris (1911) 200p.

[3] ROUBAUD, E., Les mouches tsétsés dans l'Ouest africain, Distribution géographique, Histoire, Rôle pathogène, I. La distribution géographique des tsétsés, Annls Inst. Pasteur, Paris XXXVI 10 (1915) 720-28, 1 carte h. t.

[4] DUFOUGERE, M. W., La maladie du sommeil et les trypanosomiases animales en Casamance, J. off. Afr. occid. fr. 69 (suppl.) (1912) 7p.

[5] BOUET, Contribution à l'étude des zones à glossines du Sénégal (région du chemin de fer, de Thiès à Kayes), Bull. Soc. Path. exot. IX (1916) 802p.

[6] MOULANIER, M., DIOUF, J., Dix années de lutte contre la maladie du sommeil sur la Petite-Côte du Sénégal et la région des Niayes, Bull. méd. A. O. F. IX 2 (1952) 201-38.
[7] MOREL, P.C., Enquête sur les glossines de la région des Niayes (Rapport polycopié), Lab. nat. Elev. Rech. vét. Dakar (1963) 5p.
[8] GOUZIEN, P., Répartition topographique de la maladie du sommeil dans l'Afrique tropicale française. Publication de l'Association scient. int. Agro. col. Paris, Levé édit. (1908) 26-31.
[9] GASCHEN, H., Les glossines de l'Afrique occidentale française, Acta trop. Suppl. 11 (1945) 131p.
[10] SERVICES GEOGRAPHIQUES DE L'A.O.F., Limites des aires de distribution de Glossina palpalis et Glossina tachinoides, Glossina morsitans et G. longipalpis en Afrique occidentale, 2 cartes (1949).
[11] MORNET, P., Carte de répartition des trypanosomes pathogènes des animaux domestiques en Afrique occidentale française, Bull. Soc. Path. exot. XII (1953) 308-11.
[12] POTTS, W.H., Distribution of Tse-tse Species in Africa, Directorate of Colonial Surveys, London, 3 maps, sheet 1: West Africa (1953).
[13] RICKENBACH, A., Carte de répartition des glossines en Afrique occidentale d'expression française, Off. Rech. scient. tech. Outre-Mer, Paris (1961).

BIBLIOGRAPHIE

BUXTON, P.A., The Natural History of the Tse-tse Flies, An Account of the Biology of the Genus Glossina (Diptera), London School Hyg. trop. Med. X (1955) 816p.
FINELLE, P., ITARD, J., YVORE, P., LACOTTE, R., Répartition des glossines en République centrafricaine, Etat actuel des connaissances, Revue Elev. Méd. vét. Pays trop. XVI 3 (1963) 337-48, 4 cartes.
FORD, J., The Distribution of the Vectors of African Pathogenic Trypanosomes, Bull. OMS XXVIII 5-6 (1963) 653-69.
KEAY, R.W.J., Vegetation Map of Africa South of the Tropic of Cancer, Oxford University Press, London, (1959) 24p.
MACHADO de BARROS, A., Révision systématique des glossines du groupe palpalis (Diptera), Public. Serv. cult. Companhia Diamantes Angola, Lisbonne, XXII (1954) 189p.
NEWSTEAD, Guide to the Study of the Tse-tse Flies, Livetpool School trop. Med. I (n.s.) (1924) 332p.

PANEL RECOMMENDATIONS

GENERAL

The Panel is impressed by the advances in research achieved in genetic control techniques in the last few years. It considers these techniques to be very promising and that research on these lines should certainly be continued or even expanded.

Attention should always be paid to any factors in the life history of arthropod pests which render them especially susceptible to the sterile-male technique. In this connection it is noticed that several livestock pests exhibit such factors in that they exist in relatively low numbers and/or have restricted host relationships.

The Panel considers that in the planning of research leading to control measures, consideration should be given to economic factors.

Since control of certain pests would result in a rapid increase of livestock products, the Panel lists examples of such pests that may be amenable to control by the sterile-male technique. They are:

Species with considerable research background

Dermatobia hominis - tropical ox warble
Glossina (major species) - tsetse flies
Haematobia irritans - horn fly
Hypoderma sp. - cattle grubs
Lucilia sericata - blowfly

Species with limited research background

Oestrus ovis - sheep bot fly
Stomoxys calcitrans - stable fly
Musca autumnalis - face fly
Culicoides sp. - gnats

As regards ticks, research by Galun (Israel) has indicated some promise of progress on one species, *Ornithodorus tholozani*. It is suggested that basic research leading to the application of the sterile-male technique for control of the economically important *Boophilus annulatus* complex be initiated.

The Panel recommended that at future meetings, greater emphasis be given to studies on ticks.

The Panel endorses the general recommendations reported in "Advances in Insect Population Control by the Sterile-Male Technique"[1], wherever applicable to livestock pests.

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[1] INTERNATIONAL ATOMIC ENERGY AGENCY, Advances in Insect Population Control by the Sterile-Male Technique, Technical Report Series No.44, IAEA, Vienna (1965).

RESEARCH AND DEVELOPMENT

The Panel considers that a pre-requisite to any sterile-male technique programme is a detailed knowledge in genetics, ecology and mass production. Relevant aspects of these subjects, as well as some specific examples of problems requiring investigation, are listed below.

1. Genetics

Studies on formal genetics and cytogenetics, particularly on the effect of radiation on chromosomes; isolation and description of radiation-induced mutants that can be used as markers; population studies of laboratory and field strains.

Examples:

(a) Studies of the effects of radiation on cytogenetics of Dermatobia.
(b) Studies of sterilization treatment on major vector species of Glossina.
(c) Studies of the genetic adaptation during colonization of Glossina.

2. Ecology (and other biological aspects)

These studies would include population dynamics, species behaviour with special reference to mating and extent of dispersal, and studies of microclimates.

Some aspects of physiology such as sudies of pheromones and other attractants, availability of proteases, etc., can contribute to a more cogent approach to the use of the sterile-male technique.

Examples:

(a) Assessment of Dermatobia populations in typical habitats.
(b) Development of survey techniques to estimate the size of natural Glossina populations and to detect Glossina at low population densities.
(c) Studies of the dispersal in the field of laboratory-reared Hypoderma using isotope-tagged or otherwise marked insects.

3. Mass production

Investigation of optimal dietary and environmental requirements, evolving into the development of natural and artificial media studies of the relation of innate reproductive capacity to numerical requirements.

Examples:

(a) Development of methods of artificial feeding of blood-sucking insects, particularly Glossina.
(b) Development of in vitro procedures for rearing Dermatobia larvae.

(c) Improvement of rearing methods for horn flies, with emphasis on the production of fully competitive males.
(d) Development of rearing methods for Boophilus spp.

COMMUNICATION BETWEEN SCIENTISTS

Improved communication in research on livestock pests is needed. The following recommendations are made:

(1) Every effort should be made to achieve full exchange of information between workers concerned with the genetics, ecology and rearing of arthropod pests.

(2) The first step in promoting research on the problem outlined above should be the establishment of an internationally co-ordinated programme of research on the use of the sterile-male technique to control animal insect pests. The Panel therefore recommends the adoption of the proposal given in detail in Annex 1. Further, participants in such programmes should meet for co-ordinating sessions at intervals of about two years. Further, the proceedings of this Panel should be published as an Agency publication in the Panel Proceedings Series.

(3) The Panel fully recognizes the invaluable service given to entomologists dealing with radiation and radioisotopes by the Agency's bibliographic series "Radioisotopes and Ionizing Radiations in Entomology". The Panel recommends that this service of the Agency be continued and expanded.

JOINT FAO/IAEA DIVISION OF ATOMIC ENERGY IN AGRICULTURE

ANNEX 1

PROPOSAL FOR A CO-ORDINATED PROGRAMME OF RESEARCH ON THE USE OF RADIATION TO CONTROL ANIMAL INSECT PESTS EMPHASIZING GENETIC ASPECTS

PURPOSE

To establish a mechanism for international co-ordination of the work of some of the more active research workers in radiation control and eradication of insect pests to animals.

To foster more rapid progress in improvement of methods of producing mutations and using them in control and eradication programmes of animal insect pests by:

Concentrating efforts to identify significant problems which hinder progress, and to design and co-ordinate experiments to find solutions to such problems;

Selecting animal insect problems which lend themselves to approach by mutation methods, and finding effective procedures for applying them;

Providing information concerning efficient mutagen treatment techniques, encouraging the application of efficient techniques, and providing to the fullest possible extent guidance for projects in this subject;

Establishing uniform procedures for reporting, and analysing research data;

Promoting more rapid and direct exchange of ideas and experimental material;

Rendering such assistance as is possible to help workers in the field obtain financial support for their research projects.

SCIENTIFIC OBJECTIVES

To improve methods of controlling the mutation process(es), the basis of the sterile-male technique to control insect pests;

To study methods of more efficient use of mutagens for production of the maximum number of genic or chromosomal mutations;

To assess long- and short-term effects of chemosterilants on insects and hazards projected to other organisms;

To promote genetics and ecology in radiation biological studies for insect control;

To determine the effectiveness on insect population performance of combinations of factors (e.g. radiation and chemosterilants) at sterilizing and sub-sterilizing levels;

To initiate or amplify the sterile-male technique research against promising candidate species of economic insect pests on animals;

To evaluate techniques such as hybridization versus selection in sterile-male research;

To contrast the economic advantages of the sterile-male technique with those of insecticide and biological control.

MECHANISM

Establishment of a co-ordinated research group for which Agency sponsorship would be provided by Research Agreements or Research Contracts under the technical supervision of the Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture.

Since the research projects carried out by the participants are for the most part current and funded from various sources in Member States of the Agency, the Agency itself does not normally contribute funds to these separate projects. This research is, therefore, usually donated to the Agency to form part of its programme on a "cost-free" basis.

The Agency's function is primarily to serve as the co-ordinator and home base for this programme. The co-ordination is carried out chiefly at annual panel meetings sponsored by the Agency. At these meetings the participants report on past activities performed under the programme, outline their ideas on future work, formulate the joint approach, and plan for the coming year by designing joint projects or by parcelling out specific tasks.

The papers read or submitted and the recommendations agreed on are published.

Further co-ordination is achieved by circulating an Information Circular on Radiation Techniques and their Application to Insect Pests (lists and summaries of recent papers, significant new research findings and other items of interest), maintaining direct contact with participants, publishing a comprehensive bibliography of radiation entomology containing world-wide references, abstracts, tables on sterilization, dispersion and insecticides, and corporate and personal author indices, and providing such services to participants of which the Agency is capable.

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