AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D. C. 20523
BIBLIOGRAPHIC INPUT SHEET

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Batch 72

1. SUBJECT CLASSIFICATION
A. PRIMARY: Serials — Y-AL72-0000-G100
B. SECONDARY: Food production and nutrition--Pests of animals--Africa

2. TITLE AND SUBTITLE
Research on the sterility method of tsetse fly control; progress report, June-Dec.1964

3. AUTHOR(S)
(101) U.S. Agr.Research Service

4. DOCUMENT DATE: 1965
5. NUMBER OF PAGES: 6p.
6. ARC NUMBER: ARC

7. REFERENCE ORGANIZATION NAME AND ADDRESS
USDA/ARS

8. SUPPLEMENTARY NOTES (*Sponsoring Organization, Publishers, Availability*)
(Research summary)

9. ABSTRACT

10. CONTROL NUMBER
PN-RAB-728

11. PRICE OF DOCUMENT

12. DESCRIPTORS
Tsetse flies Africa

13. PROJECT NUMBER

14. CONTRACT NUMBER
PASA RA-1-00 Res.

15. TYPE OF DOCUMENT

AID 590-1 (4-74)

RESEARCH ON THE STERILITY METHOD OF TSETSE FLY CONTROL OR ERADICATION

THIRD PROGRESS REPORT (JUNE 1964-JANUARY 1965)

Research on Chemosterilization of the Tsetse Fly, Glossina morsitans orientalis - ARS

Considerable progress has been made during the six-month period from June 1964 to January 1965 on methods of chemically sterilizing tsetse flies. Research was concentrated on four different methods of administering chemosterilants to the tsetse fly, Glossina moristans and included also some chemosterilization studies with Glossina pallidipes and related studies on biology, mating, and behavior. These studies, summarized below, would have been impossible without the help of the Agricultural Research Council (ARC) of Central Africa which supplied approximately 63,000 field collected tsetse fly pupae from which 40,000 adult flies emerged for testing.

G. morsitans males, injected with 1 ul. of aqueous sterilant solution, were completely sterilized with 1 ug. of tepa and 5 ug. of metepa; apholate and hempa produced high mortality without attaining complete sterility.

Two day old G. morsitans males were completely sterilized by exposure of 15 to 240 minutes to residues of 10 and 50 mg/sq ft of tepa on glass. Exposures of 60 minutes or less to 10 mg/sq ft apparently did not reduce male longevity, although 240 minute exposures reduced the longevity of virgin males by 25% and mated males by 33%. Exposure for 24 hours caused complete male mortality within 2 days. Males and females exposed to 10 mg/sq ft for 15 or 240 minutes, when 0 to 24 hour, 1 day, or 2 day old adults, were completely sterilized. Emerging males, and females given ad libitum exposures to 10 or 50 mg/sq ft were completely sterilized.

Two and 4 hour exposures to 10 mg/sq ft of metepa caused a high degree of sterility in 2 day old G. morsitans males, but reduced the longevity of virgin males by 12% and mated males by 17%. Similar exposures to apholate produced inconsistent male sterility at 10 and 100 mg/sq ft. Hempa failed to sterilize but caused fumigant toxicity at 50 and 100 mg/sq ft.

G. morsitans males exposed for 4 hours to 10 mg/sq ft of tepa or metepa on glass failed to recover fertility during a 5 week test period, at the end of which the males were 41 days old. At this age both the treated and untreated males, having already copulated several times, exhibited a marked reduction in ability to successfully fertilize females.

Laboratory and field cage tests indicated that the sexual competitiveness of G. morsitans males was not reduced by a 4 hour exposure to residual deposits of 10 mg/sq ft of tepa on glass.

G. morsitans adults which emerged within two weeks after being dipped as pupae in 5% tepa solutions were sterile, while later emergees were only partially sterilized. Those from pupae washed 24 hours after the 5% tepa dip were fertile. Metepa at 5% caused complete sterility by the pupal dip. Lower dosages of metepa or tepa were less effective. Apholate and hempa dips produced no sterility.

Third Progress Report on the USDA/AID Research Project concerning Tsetse Fly Control

The attached technical progress report indicates excellent progress and the feasibility for continuing the work. There are a number of technical terms used which might be confusing to some readers. Therefore, a glossary of technical terms, in the sequence they appear in the text, follows:

pupae: intermediate stage between young and adult (similar to cocoons)

1 ul.: 1 micro-liter or 1 millionth of a liter
5 ug.: 5 micro-grams or 5 millionths of a gram

tepa, metepa, and apholate: complex compounds containing the aziridinyl ring (used in the dry cleaning industry)

hempa: similar to the other three compounds but without the aziridinyl ring, which presumably makes it safer to be handled by humans.

ad libitum: allowed to feed freely (on guinea pigs)

eclosed: emerged from the pupae (cocoons)

larviposition: deposition of larvae which were hatched from eggs within the female

spermathecae: an organ in the female fly where sperm are stored.

in copula: remain mated

cattle to act as foci: using cattle as an attractant for the flies.

tenerals: non-fed flies

mopane: indigenous tree cover

Fly-rounds: a method for measuring the natural population of flies by leading an animal around a specific area to attract flies.

ant-bear hole: the ant-bear is an animal which excavates holes somewhat like a woodchuck does

diurnal activity: activity during daylight

David G. White, TCR/RCD
March 9, 1965

Preliminary tests in the wind tunnel resulted in complete sterility of G. moristans males following exposure to 5% tepa.

The average reproductive capacity of 25 pairs of untreated G. morsitans adults caged for a period of 4 weeks under standard test conditions was 28 pupae, from which 26 adults (93%) successfully emerged. Mortality prior to the initiation of the test cages was negligible and survival over the 4 week test period was 72% and 77% for males and females, respectively.

Seventy-six percent of G. morsitans females held at 79±2°F had eclosed by the 24th day after larviposition while 91% of the males emerged later, suggesting a possible method of separating the sexes in the pupal stage.

A high proportion of G. morsitans females accepted a second mating when the second opportunity came soon after the first mating, but only a low proportion did so when the time lapse was as much as 3 to 4 days. Untreated males were capable of inseminating an average of 6.6 females. Males exposed for 4 hours to residual deposits of 10 mg/sq ft of tepa on glass inseminated an average of 7.7 females.

Regardless of the duration of copulation, sperm from G. morsitans males was transferred to the female predominantly during the final stages of copulation. Up to 5 mintes elapsed after the natural cessation of copulation before normal amounts of sperm were detected in the spermathecae, and 10 to 60 minutes elapsed before the sperm mass assumed its normal characteristic position and appearance in the spermatheca. No differences were seen in the behavior of sperm from untreated and treated males. Untreated males remained in copula for an average of 123 minutes and treated males 134 minutes.

Exposure of G. pallidipes for 15, 60, or 240 minutes to residual deposits of 10 mg/sq ft of tepa on glass caused high male sterility. Although the sterility was permanent, this species was less tolerant to tepa than G. morsitans and treated males exhibited a sharp reduction in male longevity, sexual vigor, and competitiveness. Untreated males inseminated an average of 13.8 females while treated males (4 hour exposure) inseminated 8.8.

A high degree of sterility was achieved in G. pallidipes males and females exposed as pupae to a 5% tepa dip.

This research demonstrates the feasibility of sterilizing tsetse flies chemically. However, further research will be undertaken on the best method of sterilization and the behavior and mating competitiveness of sexually sterile males.

Mass Rearing, Biological, and Ecological Studies - ARC

I. Introduction

During the period from June 1964 to January 1965 livestock have been well established at the Chirundu Field Station, the majority of the buildings are completed and experimentation has begun.

2. The Station

Facilities for personnel at the Chirundu Station including quarters for two Experimental Officers and two Field Assistants, a small visitor's quarters, a combined office and small laboratory building, a storeroom, and a workshop and vehicle shelter have been completed. Water is available in these quarters as well as at the animal paddocks.

Cattle and sheep which are now maintained at the Chirundu Field Station are doing extremely well; however, their maintenance requires prophylatic drug treatment because the Station is located in an area of heavy trypanosome risk.

One 5-ton international lorry was lost in an accident at the Field Station. A replacement is being obtained with insurance money. Although all vehicles supplied from the United States seemed well suited to the tasks for which they were purchased, transportation has been a problem. Operating over very rough terrain, breakdowns are frequent. Replacement parts and professional mechanics capable of servicing these vehicles are not available or are difficult to obtain.

3. Staff

Mr. John Ford, Principal Investigator for the Agricultural Research Council of Central Africa (ARC) resigned his position as leader for ARC research. He has been replaced by Mr. Godfrey J. W. Dean who will devote all of his time to this project and by Mr. R. Phelps who will assist in this project. These two scientists are familiar with tsetse fly research and will be an excellent addition to the research staff working on this research proposal.

4. Experimentation

The starting point of this project was the suggestion by Dr. E. F. Knipling that domestic animals under drug protection might be used to build up a population of higher density than is commonly observed in nature, and that from this population large deposits of pupae might be harvested. This idea, developed in conjunction with the stationary bait animals catch technique now being used by Mr. Pilson, provides an entirely new opening for field research on tsetse fly ecology.

Although the first experimental attempt to contain and produce a high density population by use of an open cage failed, it did, nevertheless, provide evidence that the basic idea of using cattle to act as foci for aggregation formation was sound.

The cage experiment was designed on the assumption that a high wall would prevent the escape of the introduced flies, which, initially of course, were all tenerals. It was assumed that these flies would feed on the oxen inside the cage and, since a proportion of them would be marked while feeding (and assuming also that the females would produce a second generation and so on) that the proportion of recaptures of marked flies would eventaully reach a constant level related to feeding and marking frequency and to population size. Catching, marking, release and recapture as a bait ox outside the cage would provide the control.

Results obtained in the cage experiment are summarized as follows:

(a) The cage did, in fact, provide an obstacle to fly movement in that it prevented flies from entering (to some degree although not entirely). Flies seeking to enter were hungry flies moving at little above ground level. The cage was less of an obstacle to "sexually appetitive" males.

(b) Teneral flies newly emerged from pupae introduced into the cage fed on the oxen inside the cage and, having fed, ascended the trunks of the trees and then moved out of the cage at top canopy level. This is easier to imagine at the beginning of the experiment, when the mopane still had a good leaf canopy, than at the end when it was leafless. Mr. J. Condy, of the Veterinary Research Laboratory, has made the useful suggestion that the cage experiment might be done again in vegetation which was known to support a tsetse population, but which is less tall than the surrounding cage wall. A G. pallidipes thicket population suggests itself.

(c) The daily presence in the cage of four oxen provided an "attraction center" for the natural ambient tsetse population which formed an aggregation around the cage of hungry flies. This was manifested firstly by the larger catch with higher female percentage at the outer ox, secondly by the failure of the catch at the outer ox to diminish in the same way as did that inside after cessation of emergence from the introduced pupae and finally, by an unexpected build up of G. pallidipes.

(d) The emergence of some 2500 flies built up the cage population and, because it quickly escaped, also augmented, for a time, the natural population of the area around the cage. We have no idea of the availability of G. morsitans in the Zambezi Valley, but if it were about 10 percent, the returns for September and October of No. 1 and No. 2 Fly-rounds, suggest a population density in the cage neighborhood of between 4 and 5000 flies per square mile. The emergence of 2500 flies might therefore be expected, for a short time, to have a measurable effect on local population density. The catch increase at the outer ox between the 10th and 24th of September could be attributed partly to this and partly to the formation of the aggregation noted in (c) above. But, of course, the availability may be more, or much less, than 10%. There is little point in futher speculation along these lines.

(e) It is worth noting that Mr. and Mrs. Pilson in a recent departmental memorandum give data (used by them for another purpose) which support the idea that a standing ox will form an attraction center about which flies will aggregate. They had four oxen under simultaneous observation on five occasions between the 12th and 21st of October, inclusive. The total of catches from all four oxen on the 12th was 151 G. morsitans and this total increased in each of the succeeding three counts to 311 on the 19th. On the 21st it was 291. Mr. Pilson has pointed out to me in conversation that one ox, in mopane woodland, did not show this marked increase. If, therefore, we consider the other three oxen (in riverine Brachystegia and Combretum communities) the relative increase is even more striking and is continuous from a total of 109 on the 12th to 260 on the 21st.

(f) It was concluded that the build up of the catch inside the cage was due to the newly emerged flies; of that outside to these flies as well as to the formation of an aggregation around the attraction center inside the cage (query - through the medium of the scent?). With the dispersal of the

introduced population, the catch inside the cage rapidly fell; but that on the outside remained relatively high.

(g) A proposed cattle grid experiment will be an attempt to repeat these observations under somewhat different conditions and, by using a number of animals distributed over a relatively large area, to build up a true population as opposed to merely creating an aggregation within an existing population.

It was thought that the pupae introduced to the cage were becoming overheated and some attention was paid to the problem of cooling the pupa tray. Later, because a cool relatively humid refuge was an essential part of the "furniture" of the small cage used for testing laboratory flies by Dr. Dame, some investigation of ant-bear hole pupa sites was carried out by Mr. Wilson. He found, by placing recording thermohygrographs in these holes that their air temperature varied between 23 and 27°C and humidity between 70 and 80%. This, of course, is a very much more equable climate than that in the outer air which, in November, when these observations were made, showed daily temperature variations in a screen between 17.1 and 41.5°C, with humidity between 36 and 85%. Mr. Wilson later constructed artificial "ant-bear holes" which gave temperatures varying between 23 and 28.5°C and relative humidities from 67 to 79%.

Experiments involving "fly-rounds" and "stationary bait animal catches" has been initiated to determine biological and ecological factors associated with tsetse flies at the Chirundu Field Station. Principal objectives of the studies are determining the diurnal activity of the flies and population trends and locations.