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The tsetse fly, Glossina spp. has been a scourge across the middle of the continent of Africa for centuries. A blook-sucking insect, it feeds generally on warm-blooded animals, principally larger wild game, domestic livestock, and man. The fly serves as an intermediate host for the trypanosome which causes the illnesses known as sleeping sickness in man and nagana in domestic livestock; many wild animals have developed immunity to the effects of the trypanosome. The illness causes a general wasting away and lethargy in both man and domestic livestock, often resulting in death. Finding an appropriate method for control of the tsetse fly would have great economic importance in Central Africa.

This study deals with examination of a proposed field test in Tanzania to demonstrate control of one species of the tsetse fly, Glossina morsitans, by the sterile insect techniques (SIT). The technique involves flooding a land area with a dominating number of sterile male flies whose matings with fertile females will produce no progeny. Theoretically, over time, with sufficient release of sterile males, the population of indigenous flies should reduce to nearly zero. If feasible economically and biologically, this kind of insect control represents an ideal control technique.

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Considerations in the Design of a Field Trial for Tsetse Fly Control

Final Report

by

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PREFACE

The tsetse fly, Glossina spp. has been a scourge across the middle of the continent of Africa for centuries. A blood-sucking insect, it feeds generally on warm-blooded animals, principally larger wild game, domestic livestock, and man. The fly serves as an intermediate host for the trypanosome which causes the illnesses known as sleeping sickness in man and nagana in domestic livestock; many wild animals have developed immunity to the effects of the trypanosome. The illness causes a general wasting away and lethargy in both man and domestic livestock, often resulting in death. Finding an appropriate method for control of the tsetse fly would have great economic importance in Central Africa.

This study deals with examination of a proposed field test in Tanzania to demonstrate control of one species of the tsetse fly, Glossina morsitans, by the sterile insect technique (SIT). The technique involves flooding a land area with a dominating number of sterile male flies whose matings with fertile females will produce no progeny. Theoretically, over time, with sufficient release of sterile males, the population of indigenous flies should reduce to nearly zero. If feasible economically and biologically, this kind of insect control represents an ideal control technique.

FOREWORD

This report is submitted to fulfill the requirements for the study "Redesign Field Trial for Tsetse Fly Control," Work Order No. 11 of Contract No. A.I.D./CM/otr-C-73-198, undertaken for The Agency for International Development, through the Office of Agriculture, Technical Assistance Bureau. The study was under the general supervision of Mr. Albert L. Brown, Vice President, American Technical Assistance Corporation (ATAC), a subsidiary of the General Research Corporation (GRC).

Agency for International Development liaison and supervision for the study was provided by Dr. Nels Konnerup, A.I.D./Washington, TA/AGR. In addition, useful information about the study was furnished by Dr. D. Leroy Williamson, Project Leader, Tsetse Research Unit, Agriculture Research Service, U. S. Department of Agriculture, Tanga, Tanzania.

The study was conducted over the period August to November 1974. Mr. Howard C. Olson, Member of the Technical Staff, ATAC/GRC was Principal Investigator. Dr. Ronald A. Ward, Supervisory Entomologist, Walter Reed Army Institute of Research, Washington, D. C., and Dr. Earl W. Kehrberg, Department of Agricultural Economics, Purdue University, West Lafayette, Indiana were members of the study team. Appendix A of the study was prepared by Mr. Floyd I. Hill, Member of the Technical Staff, GRC.

A draft of this report was reviewed by a number of persons especially knowledgeable of the tsetse fly and its control. Their comments and observations were incorporated into this volume. The reviewers were:

Dr. David A. Dame, Director, Insects Affecting Man Research Laboratory, Agricultural Research Service (ARS), U. S. Department of Agriculture (USDA), Gainesville, Florida; also Technical Advisor, Tsetse Fly Project

Dr. Anthony M. Jordan, Director, Tsetse Research Laboratory, Department of Veterinary Medicine, Bristol, England

Dr. Leo A. LaChance, Research Leader, Radiation Biology and Insect Genetics, Metabolism and Radiation Research Laboratory, ARS/USDA, Fargo, North Dakota

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Dr. Claude H. Schmidt, Director, Dakotas-Alaska Area, ARS/USDA, Fargo, North Dakota

Although many persons have contributed toward the preparation of this report, responsibility for errors or omissions rests with the authors.

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CONSIDERATIONS IN THE DESIGN OF A FIELD TRIAL FOR TSETSE FLY CONTROL

I. INTRODUCTION

A. BACKGROUND

This contract effort was initiated at the request of the U. S. Agency for International Development, Office of Agriculture, Technical Assistance Bureau. It is a short term study dealing with the control of the tsetse fly by the sterile insect technique (SIT). In the study, technology for rearing tsetse flies in the laboratory has been surveyed, including sterilization of male flies, optimum methodologies for producing adult flies that will approach in viability tsetse flies found in their natural environment, improved efficiencies in rearing, and reduced costs in rearing. In addition, the probable site in Tanzania for a sterile male release experiment was visited.

While the rearing of tsetse flies in the laboratory has many objectives from a scientific standpoint, a principal interest at this time is the laboratory rearing of flies in numbers sufficient to be able to conclusively establish by field experiment the effectiveness of the sterile insect release technique as a method to control and eradicate from a geographic area the species of fly released. Thus rearing large numbers of flies is a prerequisite to large scale field experimentation and will be commented upon in the context of the probability of being able to carry out large scale sterile insect release experiments under various resource and time restraints.

The laboratories at which rearing is underway that were visited in the course of this contract, and information from which forms the basis for most of the discussion concerning rearing, were the Tsetse Research Project, Tanga, Tanzania, the International Atomic Energy Agency Tsetse Laboratory at Seibersdorf, Austria, and the Tsetse Research Laboratory, Langford, Bristol, England. The basis of comments relative to large scale field experimentation is information available about the probable experimental site, the experiences of other investigators in such research, and the logic of experimental control generally.

B. PURPOSE

The report has three main objectives:

1. To explore and integrate factors that must be taken into account preliminary to mounting a full scale test for tsetse fly control and possible eradication by the SIT;

2. To determine the critical parameters for control in a field test for tsetse fly control on a probable test site in Tanzania;

3. To visit the probable test site and develop a preliminary field test design.

A bias of sorts will be evident in the report: It is assumed that sufficient effort already has gone into rearing methodology for the particular species of fly under study (*Glossina morsitans*), and that commitments between USAID and the government of Tanzania are of such duration and consequence that it is nearly imperative that a controlled field test eventually result. The vagaries of tsetse population dynamics in the wild state, the vicissitudes of rearing, and the uncertainties of funding all pose difficulties in planning; they require that options be developed whose objectives may be somewhat less in scope than the desired full scale field experiment, so that, no matter what, a well controlled field test will be carried out in the time frame planned. In other words, a sound sterile male release experiment will have been conducted, although the land area from which the species will presumably have been eradicated may be less than originally anticipated.

C. CONTROL METHODS FOR TSETSE FLY

1. General

Over the years a number of techniques have been employed for tsetse control; none alone has been eminent in its success. What works with one species may not work with another; what is effective in one geographic or ecological niche may not be so in another. By and large it appears that starving the fly through removal of its source of mammalian hosts may be more successful than any other method, but it does not have general public approval. Over the past 30 years, insecticides have been used

successfully. The lesson to be learned is that successful control, considering ecological, political, and practical logistical constraints is likely to involve several techniques used in sequence, or in combination. Various control measures are reviewed in this report because they have implications for barriers, which have been postulated as necessary to an experimental demonstration of tsetse fly control, such as that planned for the AID/Tanzania project. The principal methods that have been used more or less successfully will be described briefly.

2. Clearing

Clearing is a technique used for the destruction of tsetse habitat through the removal of woody vegetation. In the past this procedure was used in both East and West Africa prior to the development of organic insecticides and when large crews of workers could be recruited at low cost to clear the land.

The purpose of clearing is to provide a barrier to tsetse movement; it consists of a zone completely devoid of suitable habitat between the land to be reclaimed from tsetse infestation and that remaining infested.

Clearing may be accomplished by hand-cutting with hand-saws, ring-barking trees, burning, spraying herbicides, or using bulldozers or tractors with chains between them to uproot trees. Unless clearing is thoroughly accomplished, regrowth of trees occurs within 2 to 3 years, thus negating the effort. Although the use of bulldozers may disrupt the landscape in an aesthetic sense, it is probably the most economic procedure today. It should be pointed out that in most clearing operations, a certain amount of hand-cutting will still be required due to irregularities in the terrain and presence of trees with large trunks.

The width of the cleared zone varies according to the tsetse species and the area of Africa in which control is being attempted. In some instances, a cleared area of 0.5 miles may serve as a barrier. In a

large-scale control project in Uganda, Wooff* used a 4-mile wide cleared zone for cattle grazing land to be protected against reinfestation by Glossina morsitans and G. pallidipes. Using pairs of Caterpillar tractors, he was able to clear 4.2 to 9.8 acres of land per tractor-hour. (It is reported that LeRoux, in Kenya, has the most current data on speed and cost of clearing, and his advice is being sought by the Tanga project with respect to the Tanga clearing requirement.)

3. Game Destruction

Wild animals, besides being the natural source of food for tsetse flies are also the natural hosts of the pathogenic trypanosomes of domestic animals. There is much evidence that indicates that G. morsitans largely feeds on game mammals in its natural savannah habitat. Theoretically, when a game-free area of 5 to 10 miles is created, a zone is formed which will become tsetse-free. However, this is seldom practical, and with the current emphasis on conservation, it is not a recommended practice.

4. Fences

In Zambia, fences to restrict the movement of game and thus the tsetse, have been used as a form of control to prevent tsetse from advancing into cattle-grazing areas and to set up fly-free corridors for movement of cattle. Although of value, the expense of construction and fence maintenance precludes widespread use of fences in restraining game.

5. Hand-catching and Trapping

Historically, these were the two earliest methods of tsetse control. In Zululand, the use of 1000 Harris traps collected 7,000,000 G. pallidipes in a 100-mile^2 area over several years. Although this produced a reduction in population size, the species was not eradicated. Both these procedures are most effective in areas of high fly population density and where abundant manpower is available. The efficiency of trapping varies enormously from area to area, and a successful trap in one zone is often useless in another

*Wooff, W.R., "Consolidation in Tsetse Reclamation", International Scientific Council for Trypanosomiasis Research, 11th Meeting, Publ. scient. tech., Res. Commu., Organization of African Unity, No. 100, Lagos, 1967.

area. These two procedures have utility as tools for tsetse survey and population investigations.

6. Insecticides

The application of an insecticide, due to its high degree of effectiveness and relatively low cost, is the most widely used method of controlling tsetse flies today. The two most widely used procedures for tsetse work involves the application of insecticides from the air or the application of residual insecticides to vegetation from portable ground equipment.

Aerial application of insecticides has been used in a number of savannah areas of East Africa, particularly within the areas where flies are concentrated during the long, hot dry season. These are most effectively applied during the brief period of the year when the trees are leafless so that the insecticide will penetrate beneath the canopy to cover the tsetse resting sites on the vegetation. The essential features of an ultralow volume application have been developed by the Tropical Pesticides Research Institute in Arusha, Tanzania. These include:

a. Use of concentrated insecticide solutions in non-volatile oils;

b. Dispersion as medium aerosols having diameters in the range of 20 - 50 microns;

c. Insecticides highly toxic to Glossina. These include DDT, dieldrin, endosulphan, fenthion and the synthetic pyrethroids (e.g., resmethrin);

d. Volume dosages per application are in the range of 0.012 - 0.025 gallons per acre* and of active ingredients, 6 - 15 gal/a;

e. The area to be treated is covered by successive parallel flights alternately from opposite directions. Swath widths are 50 - 70 yards. Aircraft should fly as low as possible;

f. Meteorological conditions normally restrict treatment to the very early morning and late evening;

---

*In the case of resmethrin, the estimated dosage is 0.0025 gallons/acre. This insecticide has not been used in the field against tsetse flies but is extremely effective against houseflies, stableflies and many mosquito species.

g. Treatments are usually repeated, at equal intervals of approximately 2 to 3 weeks, for 4 to 8 times to effect control. For sterile male programs, insecticide applications are suggested, at about 3-week intervals*;

h. Light, but not the very light, aircraft are the most economical for operation.

All modern research using portable ground equipment is based upon the use of residual deposits of an insecticide which will remain effective for a period of 5 to 6 weeks. This is the maximal length of a pupal development period. Thus, flies emerging from pupae after the completion of a spraying operation will be killed.

A variety of types of equipment have been used for ground application of insecticides, ranging from back-pack sprayers holding several gallons to large ultralow volume spray systems mounted on trucks. Rather than attempting to spray all the vegetation with an insecticide, there is a growing tendency to selectively apply the materials based upon a knowledge of the resting habits of the fly. As an example, in portions of East Africa which are relatively dry during portions of the year, it is known that G. morsitans rests on the lowest part of the tree trunks where temperatures are cooler and the humidity highest during the hottest part of the day. So, spraying just the lower half of tree trunks with a residual insecticide such as dieldrin can control this species in this habitat. On the other hand, in Southern Nigeria where climate conditions are less severe, selective spraying of the lower half of tree trunks is not an effective control measure. This serves to emphasize the need for sound ecologic investigation prior to the start of a control program.

Essentially the same insecticides are used for ground application as for aerial spraying, except the formulations are different. With the exception of the synthetic pyrethroids, all have a residual effect.

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*The actual length of this interval may be slightly less than 3 weeks or longer than 3 weeks, depending upon the duration of the pupal stage.

7. Parasites and Predators

As Nash* notes, "The tsetse has many enemies, but none are outstanding." Predators and parasites attack all stages of the fly, but seldom are their depredations extensive enough to seriously influence the tsetse population. In the area of Tanzania where the field test is likely to occur, the Tanga project entomologist responsible for collection of tsetse pupae from the field has noted recently an apparent increase in the incidence of parasitism by a Bombyliid fly; still, the rate of parasitized pupae is less than 5 percent. In short, one may discount the control exercised by parasites and predators.

8. Sterile Insect Release

Since this whole effort is concerned with testing the effectiveness of flooding a tsetse population with a dominating number of sterile males in relation to the number of males in the wild population, little discussion need be made relative to the technique. It should be noted that de Azevdeo**, et.al., raises some critical questions concerning the cost of implementing the sterile male release approach, notwithstanding its efficiency as a control technique. His hypothetical cost study, using actual data from tsetse control by other means as compared with control by the sterile male release method, shows the sterile male release method to be extremely costly unless the population of wild flies is at a very low density. While his study is based upon laboratory costs of small scale rearing, it points up the necessity of reducing the tsetse population to low levels by means other than the sterile insect release approach before initiating the release campaign.

D. AID TANZANIA TSETSE STUDY

The AID/USDA project, "Research on Sterility Methods of Tsetse Fly Control" was initiated in FY 1965. Two major objectives needed to be met: (1) to demonstrate that the sterile insect approach would work with tsetse

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*Nash, T.A.M., "Africa's Bane, the Tsetse Fly," London: Collins Press, 1969.

**de Azevedo, J.F., et.al., "Biological Studies Carried Out with the Glossina morsitans Colony of Lisbon, III-Hypothetical Control of the Tsetse Fly in Principle Island by the Sterile-Male Technique," Andis da Escola Nacional de Saude Publica e de Medicina Tropical, II, Jan/Dz, 1968.

flies, and (2) to demonstrate that it was possible to rear the tsetse fly in captivity in such numbers as to be able to effectively "flood" a land area of significant size to control the species of tsetse in that area. The research of Dame and Ford* and of Dame and Schmidt** in Rhodesia had already shown the possibility of ridding an isolated land area of a species of tsetse by using sterile males reared from pupae collected in the field.

For several years, researchers have been perfecting techniques to rear a closed colony of the fly, wherein its full life cycle including breeding, is accomplished under laboratory conditions. A number of investigators have developed rearing methods but not all have found the key to developing self-perpetuating colonies that also rapidly increase in numbers. T.A.M. Nash and A.M. Jordan, of the Tsetse Research Laboratory, University of Bristol, Langford, Bristol, England, have been most successful in this respect, having learned the combination of environmental conditions, feeding, and handling regimen that will produce an exponentially increasing colony. The early development of rearing methodology by Nash and Jordan used G. austeni. The more recent work by Jordan has concentrated on mass colonization of G. morsitans with considerable success. The Seibersdorf, Austria laboratory of the International Atomic Energy Agency, in addition to having reared G. morsitans successfully, is currently having modest success with a small colony of G. palpalis. It is notable that different species of the same genus often have different requirements to assure successful rearing and the increase of a colony. At this point, G. morsitans is being reared most successfully.

The Tanzania tsetse rearing facility was first established at Morogoro, but was moved to Tanga at the request to the Tanzanian government before the facility reached full operational status. The Tanga facility has been

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*Dame, D.A. and Ford, H.R., "Effect of the chemosterilant tepa on Glossina morsitans Westwood," Bulletin of Entomological Research, Vol 56, pp. 649-658.

**Dame, D.A. and Schmidt, C.H., "The sterile male technique against tsetse flies, Glossina spp," Bulletin of the Entomological Society of America, Vol. 16, pp. 24-30.

operating for about 2-1/2 years. Owing to a series of personnel reassignments, accidents, and misfortunes in the rearing facility, it has not reached the level of fly production anticipated. The record of activity and improvements in establishing a thriving tsetse colony has improved markedly during this year. During the current calendar year a number of personnel vacancies have been filled and replacements made, operational procedures and controls in rearing of host animals and in feeding, maintaining, and breeding of flies have been improved, and collection of wild pupae to build up the base of the colony has been stepped up. Rearing practices are modeled after the rearing technology developed by Nash and Jordan* at Bristol; goats (and a few rabbits) are the source of blood for feeding the adult tsetse flies.

At this point, the Tanga Tsetse Research Project has a total of about 14,000 flies in the five colonies that it maintains. The goal is to increase to a stock colony of about 60,000 with an excess of 30,000 adult males per month, with the hope that this can be accomplished within the next 12 months.

Plans for the next 5 years for the Tanga facility are illustrated in Table 1. The work currently is in Phase I, and concerns development of facilities and establishment of rearing practices; economical rearing may include addition of membrane feeding, which if successfully implemented may supplant use of live host animals. Phase II (from 1975 through 1977) deals with all aspects of the project; continuation of rearing, handling techniques and methods development, and population dynamics. Using the excess stock of males produced, a number of preliminary studies will be conducted during Phase II, in developing sterilization techniques, marking of released flies, fly packaging and transporting procedures, distribution and release methods, typanosome detection, fly sampling methodologies, and so on. Phase III is planned for 1978 and 1979, and will be concerned with preparations for the field test, including insecticide treatment of the test area, tsetse and trypanosome surveys, barrier construction, and finally, the actual release of sterile male flies.

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*Dr. Anthony Jordan has served as an expert consultant to the project, and has trained several of the Tanga staff at the Bristol laboratory for periods of 3 weeks to 3 months.

Table 1

Schematic Work Plan - Tanga Facility

Activity	Phase I			Phase II			Phase III	
	FY 72	FY 73	FY 74	FY 75	FY 76	FY 77	FY 78	FY 79
Rearing								
Construction of research facility	x	x	x	x	x			
Pupal collection		x	x	x	x			
Initiation of fly colony		x	x					
Expansion of fly colony				x	x	x		
Increase host animal herd			x	x	x	x		
Construction of insectaries (3)		x	x	x	x			
Expansion of forage production			x	x	x			
Artificial membrane research (IAEA)	x	x	x	x	x			
Artificial membrane evaluation (Tanga)					x	x		
Initiate back-up colony (external)							x	
Techniques and Methods Development								
Pupal maintenance and sexing				x	x			
Identification of released flies				x	x			
Sterilization					x	x		
Quality control					x	x		
Packaging and transport of flies					x	x		
Distribution and release of flies; ground and aerial					x	x		
Assessment of sterile male effectiveness and female sterility					x	x		
Trypanosome detection					x	x		
Release of Tanga flies					x	x		
Release of membrane flies from IAEA				x	x			

Table 1, continued

Schematic Work Plan - Tanga Facility

Activity	Phase I			Phase II			Phase III	
	FY 72	FY 73	FY 74	FY 75	FY 76	FY 77	FY 78	FY 79
Field Population								
Identification of test area		x	x					
Population dynamics in test area				x	x	x		
Construction of basic research facility in test area					x			
Isolation of test area (barrier)				x	x			
Insecticide treatment of barrier perimeter					x	x	x	x
Evaluation of barrier					x	x	x	x
Trypanosome survey in test area					x	x	x	x
Release Program								
Tsetse suppression in test area							x	
Release program							x	x

Source: Draft Project Agreement (dated 28 March 1974) between AID and the Treasury of the United Republic of Tanzania, "Research on Sterility Methods of Tsetse Fly Control."

The probable test site is the Mzeri Hill Ranch, a nationalized cattle ranch about 65 air miles (80 road miles) west of Tanga. The area being considered for the test comprises 162 square miles; it is gently rolling, at elevations of 1500 to 2000 feet; rainfall averages about 20 inches per year. Ground cover is moderately sparse low ground vegetation (having been grazed fairly heavily), with shrubs 6 to 10 feet high and occasional trees up to 12 inches in diameter. G. morsitans predominates in the area, although several other species have been noted. No fly counts to determine tsetse density have been made during the term of the Tanga facility.

II. OPERATIONAL AND ECONOMIC FACTORS IN TSETSE FLY CONTROL

In an ideal research study, one would like to be able to experimentally establish each of the various significant operations and controls that are desirable in subsequent and concluding tests. It is rare, however, that one has the luxury of time and other resources to be able to establish each operation and control so explicitly. What one must do instead is to choose on the basis of what seems more probable of success those operations and controls that in the estimation and good judgment of the experimenter will result in a successful end-product. That is the condition that one faces in the current Tanzania study -- that is, it is realized that what is likely to be more successful in the way of rearing, sterilization, releasing, counting, and so on will have been decided as a matter of judgment, sometimes without having demonstrated experimentally the optimality of the choice. These operations will then be put together into what is hoped will be a reasonable and successful system for the experiment as a whole. A number of such factors need to be considered in developing the full system that culminates in the field experiment.

A. SUCCESSFUL REARING THROUGH AND BEYOND FY 75

1. Goals

As noted earlier, it is the goal of the Tanga facility to have an internal colony of Tanzanian-collected *G. morsitans* of 60,000 flies by the end of the next year. It is being presumed that this will be sufficient to provide an excess of 30,000 males per month to be available for preliminary field studies and finally for the release experiment; whether or not this goal can be reached cannot yet be ascertained. As of the first week in September 1974, the Tanga colony had about 9000* adult files distributed among five different colonies, with the following numbers of adult females in each colony:

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*At the end of September the colony totaled about 14,000, with about 1000 more in the internal colony (an increase of about 200 per week) and 4000 more in the external colonies.

Internal colony (self perpetuating)	2700
External I "	528
External II "	1768
Bristol "	608
Rabbit "	486

The Internal colony has been self-supporting during this calendar year, and there has been no new imput to it. The External I and II colonies are developed from pupae collected in the field, so will increase markedly in size as new collections of pupae are added to them. (During July and August about 10,000 pupae were collected from the field.) The Bristol colony is one that was derived from morsitans pupae from Jordan's Bristol colony. The colony was established at Tanga also so comparison could be made between the Bristol strain of morsitans and the Tanga strain. The Tanga strain is from pupae collected in the field about 50 miles to the south of the probable test site, while the Bristol strain has its origin from morsitans pupae collected in Rhodesia. The rabbit colony is one maintained to test the use of different kinds of rabbits as live host animals for fly feeding. The figures given are colony sizes in terms of female flies. In general, the size of a colony is something less than double the number of females since female tsetse tend to live longer than males.

It should be possible to use growth of the Internal colony at Tanga to make estimates as to colony size in the future; the other colonies are not necessarily allowed to hold all the new offspring produced--the Bristol colony, for example, has just recently been reduced from 1175 to 608 females. The increase in size of the Internal colony from 1 May until 3 September is plotted in Fig. 1. It is seen that the increase appears to be linear regardless of the base population. Ideally, one would like to see an exponential growth curve. Fig. 1 indicates, however, that the average increase in colony size is a little over 100 flies per week. It is puzzling that this growth curve does not show an acceleration, since there continues to be a larger and larger base of adult flies which should be producing a continual increase in numbers of flies each week.

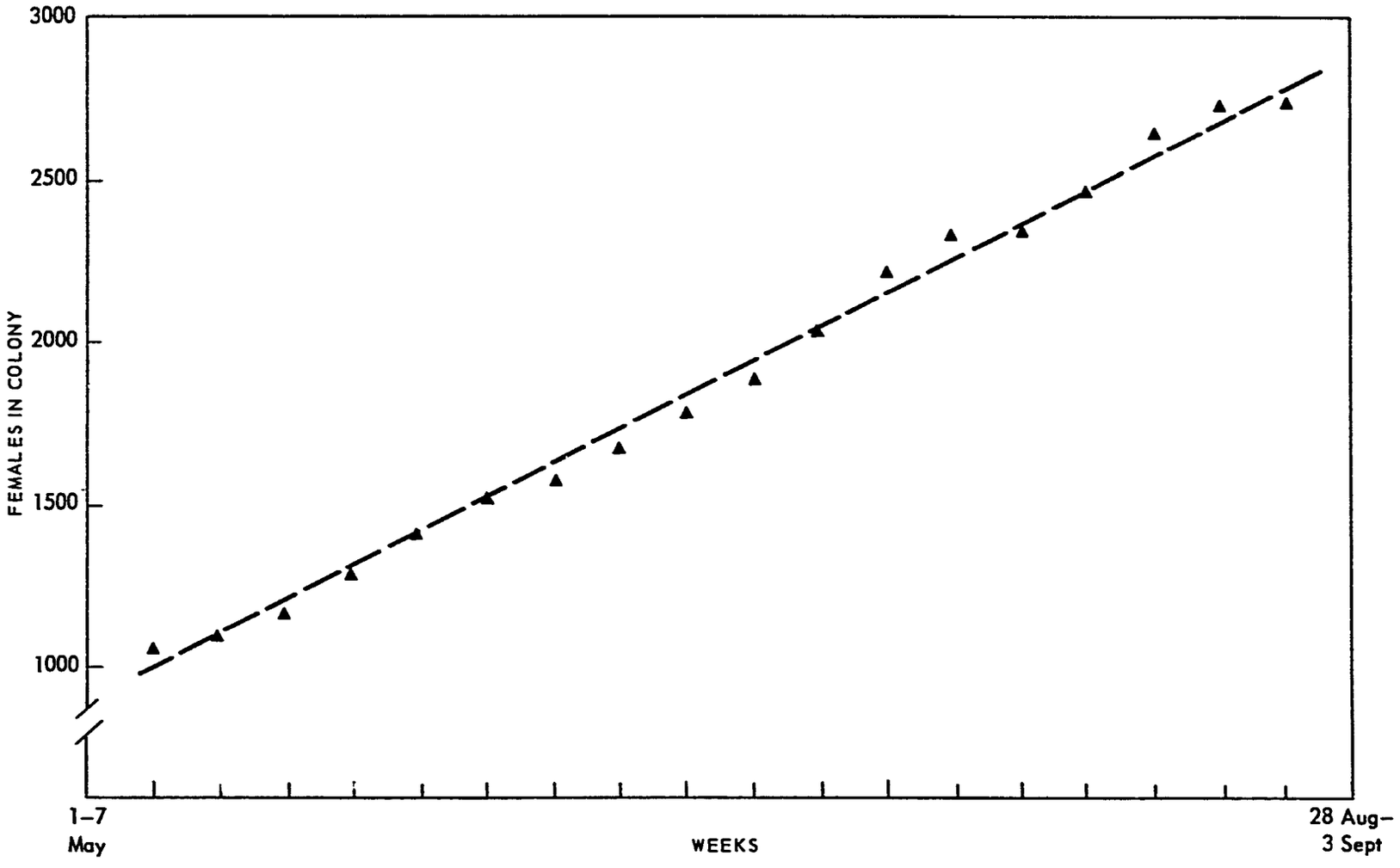

Fig. 1—Increase in Tanga Internal Colony over an 18-Week Period

If one were to use these data to predict the size of the colony in terms of female flies a year from now, it would be predicted that there would be about 5500 more females in the colony for a total of less than 9000, say 7000 males. This may not provide sufficient excess to supply the need for experimentation and release. The September results, as noted in a previous footnote, are encouraging. Hopefully, this indicates that the internal colony is beginning logarithmic increase in growth.

Increase in the size of the colony is conditional upon several different factors: the longevity of the females; fecundity of the female; and, of course, all the physical conditions of rearing (temperature, humidity, feeding, mating, and so on). In discussion of tsetse rearing with Dr. Jordan at the Bristol Laboratory, he acknowledged that the Tanga morsitans colony established at Bristol is not increasing at the rate expected based on the performance of his own Bristol morsitans colony. It is recognized that there is a difference in the origin of the pupae that are the bases for the Tanga and the Bristol colonies; while they are presumed to be identical species of the tsetse fly, they come from areas about 1000 miles apart (the Bristol colony originated in Rhodesia). Dr. Jordan expressed hypotheses that might account for differences. One, there indeed may be subtle race differences between the two colonies. Two, the Bristol flies may have become laboratory-adapted, having been reared for several years in a laboratory situation; that is, there may be a natural selection of flies who will maintain themselves better in a laboratory environment. He has preliminary data (Fig. 2)* indicating differences in the fecundity, and the longevity of the Tanga and Bristol strains. Initial fecundity, say for the number of pupae produced per female during the first 45 days, is 15 to 20 percent higher for the Bristol strain, and the disparity increases with increased age of the female. Longevity, or survival rates, show similar differences. After 100 days, only about 5 percent of Tanga colony females are still living, but 20 percent of the Bristol females are alive.

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*At this point in his investigations he is not yet willing to conclude that there are genuine differences between the two strains. While the evidence presented in convincing, it still is preliminary. It is felt, however, that it must be reviewed in the context of making decisions concerning plans for future fly rearing.

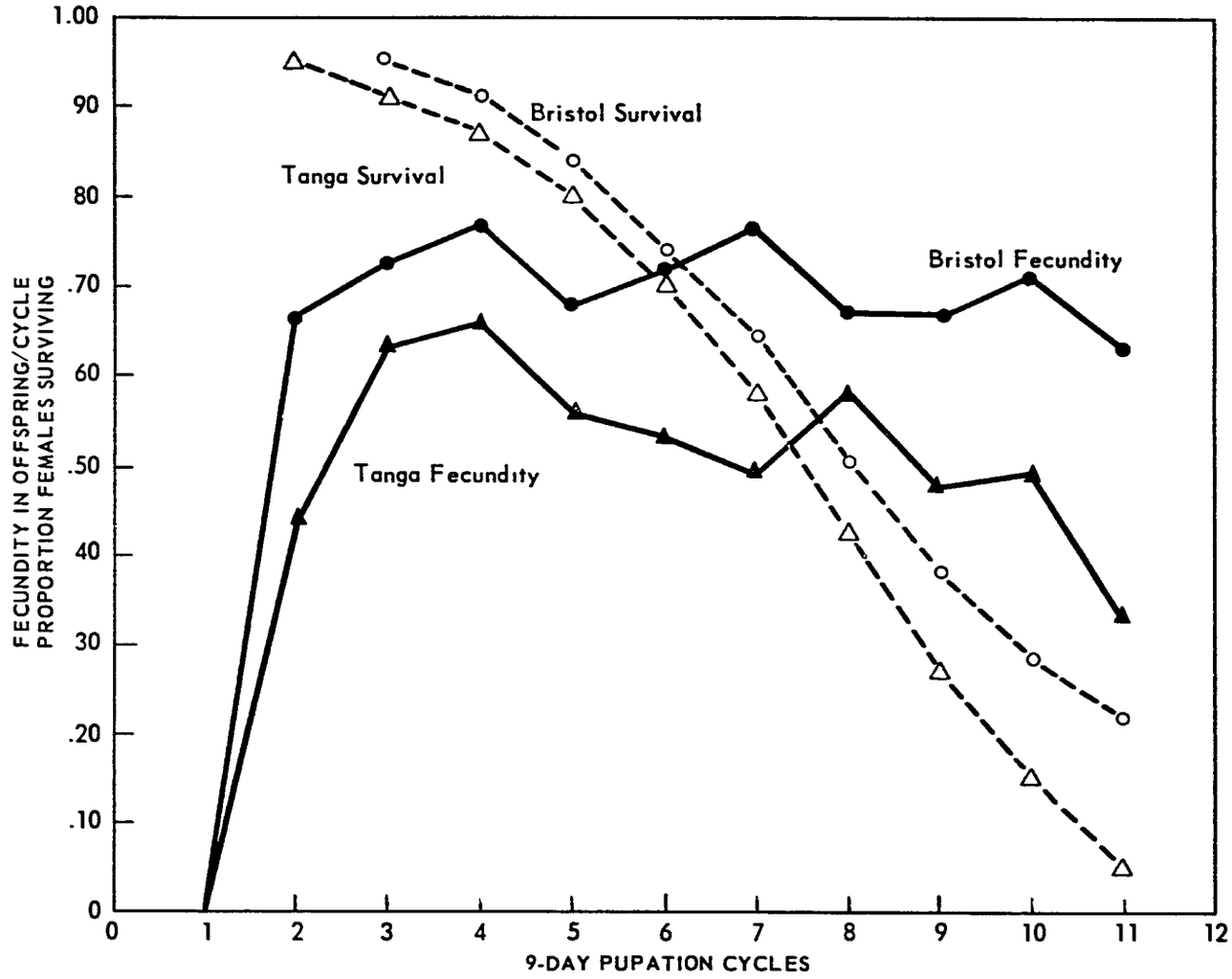

Fig. 2—Comparison of Bristol and Tanga *morsitans* at Bristol
(Data with Permission of A.M. Jordan)

In order to achieve a satisfactory growth in the colony, it is necessary that the daily mortality rate of fertilized females be 1.3 percent or less (assuming a life span of about 100 days--time to produce 10 pupae), and that the average fecundity not drop below production of 4 pupae per female. When these conditions are met the growth rate should be an accelerating one. The Tanga Internal colony had a mean mortality rate of 1.26 per day since 1 May through 3 September of this year, while the Bristol colony at the Tanga facility had a mean rate of 0.88 per day (See Fig. 3); mortality differences between the Bristol and Tanga strain are confirmed by Jordan's data (the survivability curve of Fig. 2). Combining the probabilities of the fecundity and survivability curves in Fig. 2, it is found that the Tanga colony at Bristol produced 3.12 pupae per female, while the Bristol colony at Bristol produced 4.54 pupae per female (Table 2).

It is of interest to note that if females were maintained only through the 7th pupation cycle (had opportunity to produce through about 2/3 of the life span of the longest lived), using the rates shown in Table 2, it would be possible to produce in about 72 days, 2.66 pupae per female in the Tanga strain, and 3.62 pupae per female in the Bristol strain. Thus, by terminating all females still living at about 72 days, the objective of 4.0 or more pupae per female (projecting to an optimum life span) could be reached for both strains.*

When different life spans of breeding females is considered, it probably is more convenient to express production at a daily rate. For example, differing spans convert to the following daily rates of pupae production per average female:

	Life Span 102 days	Daily	Life Span 72 days	Daily
Tanga *morsitans* (at Bristol)	3.12	.031	2.66	.037
Bristol *morsitans* (at Bristol)	4.54	.044	3.62	.050

*Jordan notes in the 1973 annual report of his laboratory that indeed he is doing this, i.e., terminating the older (less productive) female flies before they have lived out their full life span. The effect is to free rearing space for use by the younger (more productive) females.

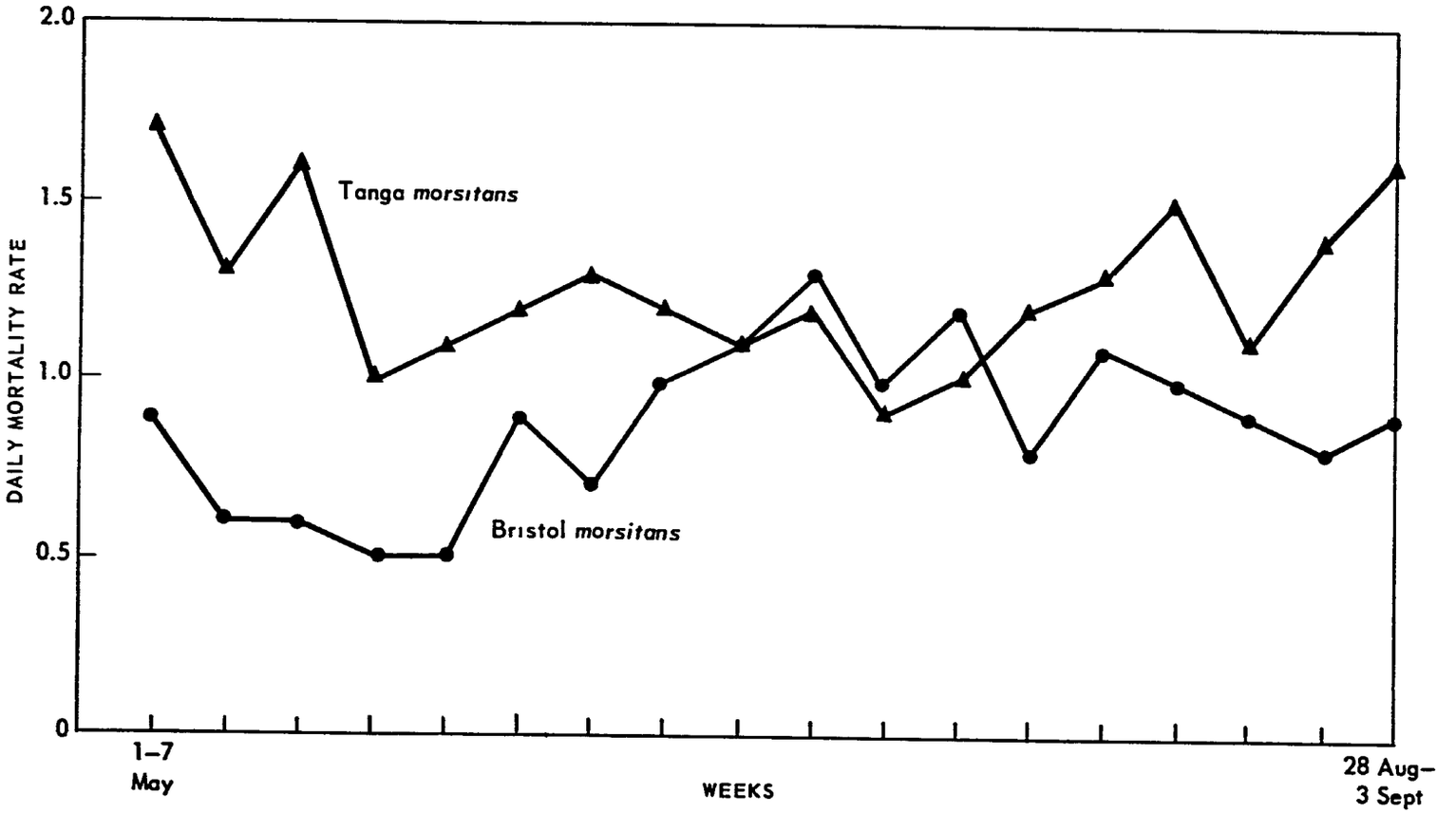

Fig. 3—Mortality Rates of Tanga and Bristol *morsitans* at Tanga

Table 2

Combination of Fecundity and Survivability Data for Tanga and Bristol *G. morsitans* Colonies at Bristol

Pupation Cycle Number	Tanga Colony			Bristol Colony		
	Proportion Surviving	Fecundity Rate	Pupa per Orig.♀	Proportion Surviving	Fecundity Rate	Pupa per Orig.♀
1	1.00	--	--	1.00	--	--
2	.95	.44	.42	.97	.67	.65
3	.90	.63	.57	.93	.73	.68
4	.87	.66	.57	.91	.77	.70
5	.80	.56	.45	.84	.68	.57
6	.70	.53	.37	.74	.72	.53
7	.58	.49	.28	.64	.77	.49
8	.42	.58	.24	.50	.67	.34
9	.27	.47	.13	.38	.67	.25
10	.15	.49	.07	.28	.71	.20
11	.05	.33	.02	.21	.63	.13
TOTAL			3.12			4.54

Factors of rearing space and requirements of newly produced male flies for breeding purposes need to be taken into account when estimating capacity of a breeding facility. It might be assumed that at any one time only about 60 percent of the rearing space in a facility can be used, since it will be noted in Table 2 that at the conclusion of the 7th pupation cycle the proportion surviving in the two colonies is 58 and 64 percent. This, however, will average out to about 20 percent of the space unused, since all cages will not be refilled at the same time. For purposes of calculation, it is estimated that 20 percent of new males produced in a colony need to be retained for breeding purposes, although it should be recognized that this figure is only speculation on the part of the authors. This means that about 80 percent of a colony is female.

Assuming then these factors:

- 20 percent of the males produced are needed for breeding to perpetuate the colony (the colony thus is 20 percent male and 80 percent female);
- 80 percent of rearing space is used at any one time;
- Pupae production is at the rate of .037 per average female per day (which assumes killing off all females after about 72 days).

A colony of 60,000 adult flies (requiring rearing space for about 75,000) would produce 1776 pupae per day, of which about half would be presumed to be male. Taking off the 20 percent for breeding would leave 710 males per day excess.

At this production rate, and using all the same assumptions as before, a total colony of about 85,000 (requiring rearing space for about 105,000 adults) would be needed to produce 1000 excess males per day.

It is evident that estimates of numbers of excess males are contingent upon various assumptions as to rates of pupal production, rearing space available, and rearing practices. For example, suppose Bristol (Rhodesian) G. morsitans, which has significantly higher survivability and fecundity rates under laboratory conditions, were demonstrated to mate satisfactorily with wild Tanga G. morsitans, and thus could be used for release purposes.

Assuming the same kind of optimization of fecundity as before (using a daily rate of 0.05 pupa per average female), 48,000 females would supply very close to the required excess of 1000 males per day. If the Tanga project is to produce a desired excess of 1000 males per day, a number of conditions must be met regarding size of colony, rearing space, strain of fly, rearing practices, and so on.

2. Rearing Sites and Backup Facilities

The two other facilities visited are rearing flies in some quantity--The International Atomic Energy Agency (IAEA) in its laboratory at Seibersdorf, Austria, and the University of Bristol in the Tsetse Research Laboratory at Langford, Bristol, England. The IAEA does not raise flies in the same quantity that they are reared at Bristol, and has never attempted mass production of tsetse; rather its concern has been with examination of various kinds of rearing and feeding techniques and with sexual sterilization methods. The laboratory at Bristol, however, has developed some self-perpetuating colonies from which pupae and adult flies are used for experimentation; excess over the laboratory's own needs are given away. The facility at the present time (as of the end of December 1973) maintained a total of about 11,000 adult flies, distributed roughly 2/5 *G. austeni* and 3/5 *G. morsitans*; with some additional equipment and some space modifications, the laboratory could accommodate 40,000 adult flies. Over the year 1973 Jordan's laboratory produced a total of 220,000 pupae. A total of 124,000 pupae or adult flies were distributed to some 48 other research institutions or investigators. These figures are cited to demonstrate that a laboratory such as that at Bristol might well serve as a backup facility for production of flies to be used for release in the Tanga experiment.

In early September 1974, the Tanga facility had one complete building for housing flies and host animals, termed Insectary No. 1. A second building is being constructed according to the same plan, and a third is planned. Insectary No. 1 is a complete set-up for tsetse fly rearing. It includes an entry chamber and shower where one may change clothes and prepare to enter the insectary proper. There is then a small laboratory

and work space, rooms for housing of flies, a feeding station, and at the end of the insectary building, a number of pens under cover for the host animals. The whole operation is efficiently operated and maintained with apparent high standards of cleanliness being observed. (Very high stardards of cleanliness are necessary in the rearing facility and among the host animals, or the growth of the colony is likely to suffer badly.) The full complement of the projected 60,000 fly colony cannot be handled until the third insectary is complete (completion is expected by the end of this fiscal year). At the present time, there is a total of about 150 goats maintained in the facility for the feeding of flies; 2/3 are being used at any one time. It has been estimated that a herd of 500 to 600 animals will be necessary for feeding the full colony, if all are maintained on goats. While goats are the main host for feeding at the Tanga facility, a small number of flies are fed on rabbits, on a trial basis.

3. Membrane Feeding

While it is still in the experimental stage, feeding flies whole blood through membranes is being experimented with both at Seibersdorf and at Bristol. It is the opinion of Dr. Lindquist at Seibersdorf and of Dr. Jordan at Bristol that membrane feeding may in the end be the more economical way to feed. At the present time, membrane feeding often is a bit hazardous and the results not always productive. It has been found both at Seibersdorf and Bristol that sterile conditions must be maintained in preparation of materials for feeding. The feeding arrangment is a series of warming plates or warming pans maintained at about 40°C. on which glass plates about 18 inches square are placed. The surface of the glass is grooved or cross-hatched. Before the defibrinated blood is poured onto the plates, the plates are flamed with a propane torch to remove any possibility of contaminating the blood. Two different membrane systems are now being used. At Seibersdorf, a silicone membrane covers and spreads the 50 cc. of blood on each plate. The membrane has been soaked in a water solution before it is spread over the glass. In Bristol a different form of a membrane is prepared which uses a combination of two membranes. The first is a very thin parafilm (m) membrane that is put over the blood to spread it, and then a very thin agar membrane (2-3% agar in water, with 5% glycerine to keep from drying) about 1.5 mm. in thickness is placed

over the parafilm membrane. The agar membranes are prepared locally the day before use; about 1/2 day of a technician's time is required to make the agar membrane. The Tsetse Research Laboratory at Bristol has just received the silicone membranes and is currently evaluating them. The membrane feeding regimen, once its details are worked out, seems to result in a speed-up of feeding time so that feeding can be done in about two-thirds of the time required using live host animals.

The number of times per week that flies require a blood meal needs to be investigated. At both the European laboratories, flies are exposed to food 6 days a week. There is evidence that an adult in its natural habitat feeds ordinarily only about every other day. It may well be that feeding can be reduced to 5 days/weekly. The time saving resulting from perfected membrane feeding, and the possibility of reduced numbers of feedings, suggests that feeding, which is a very time-consuming aspect of the rearing process, might be reduced. Whether there are differences in personnel required - animal vs. membrane feeding--is not known.

Whether or not membrane feeding could be introduced at the Tanga facility at this time is problematical. All of the Tanga facility expertise is now with the use of host animals; with the large staff of less skilled and less well-trained local personnel there is some question whether membrane feeding would be successful at Tanga. Additionally, the source of blood could be a problem at Tanga, considering the present requirement for horse or pig blood from animals free of disease. It would seem desirable first that the Tanga facility expand its present capability to the point that is has demonstrated that it can maintain a large colony of flies on live host animals, as has been projected, before any changes in feeding regimen are attempted.

There is a complication in feeding knowledge as to what type of blood is best. Horse blood is being used at the Seibersdorf laboratory, pig blood is being used in Bristol. After a period of time data will be compared to determine if there are significant differences in rearing effectiveness between the two types of blood.

4. Personnel Required

The availability of trained personnel is likely to be a continuing problem at the Tanga facility. The present staff of about 65 personnel at Tanga will need to be doubled by the time the facility reaches its full strength and a colony of 60,000 flies is being maintained. During FY 76 and FY 77, when the project reaches its full strength, 158 Tanzanian employees and 6 full-time AID employees are projected. It would appear wise to begin now to intensify recruitment for the future expansion, especially since even now the government of Tanzania has not furnished as many persons as it had agreed to.

5. Proposed Expenditures

Expenditures proposed for the next three years (Table 3) from AID and the Tanzanian government combined approximately $0.5 million a year, with about 2/3 of the total amount coming from AID and the remaining 1/3 coming from the Tanzanian government. Both Tanzanian government and AID costs are heavy with personnel costs. The Tanzanian budget includes some relatively small expenditures for laboratory buildings and vehicles and in FY 75 a proportion roughly matching the personnel expenditures for housing, equipment and supplies, travel and other non-capital expenses; a significant item of the non-capital expense is $55,000 over a 2-year period for bush clearing of the probable test site. AID expenditures are roughly half for AID personnel, and the remainder for equipment for the laboratory and field housing for AID personnel. The miscellaneous item includes aircraft hire for survey, transport and release of flies, and $25,000 for a continuing subcontract with IAEA.

For an operation the scale of that described in the project plans, the budget is not magnanimous.* All in all, it would support six AID personnel on a permanent basis, plus the numbers of Tanzanian government personnel noted earlier. There is little room for error or misjudgment. This is especially critical in view of the fact that the whole operation

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*In actuality, the FY 75 proposed budget is not being met, as the Tanzanian government is falling about $90,000 short on its commitment. Hope has been expressed that additional funds for the project might be secured from the Organization for African Unity.

Table 3

Summary of Projected Expenditures[a/]
($000)

Category	Tanzania FY 75	Tanzania FY 76	Tanzania FY 77	AID FY 75	AID FY 76	AID FY 77
Personnel	69.6	100.2	100.2	193.4	227.0	201.6
Capital Expenditures	28.6	13.3	--			
Non-Capital Expenditures	66.0	69.8	42.5			
Equipment and Supplies				65.7	54.9	54.6
Laboratory Facilities and Housing				45.9	15.3	--
Miscellaneous				15.8	22.3	25.3
Overhead, USDA Services				55.1	64.1	57.8
TOTALS	164.1	183.2	142.7	375.9	383.6	339.2

a/ From Draft Project Agreement between AID and the Treasury of the United Republic of Tanzania, March 28, 1974.

hinges on the ability to rear significant numbers of high quality male flies for release purposes. It is suggested that considerable attention should be given to backup facilities for rearing in case the facility at Tanga is not able to produce flies in the numbers expected. It is seen from Figure 1 shown earlier, that unless there are changes in rearing practices or strains of flies reared, there is some doubt that the Tanga facility alone will be able to rear flies in sufficient numbers to meet the demands of the experiment.

6. Rearing Alternatives

If indeed the requirement is for an excess of 30,000 male tsetse per month for experimental and release purposes (which of itself may be an underestimate of requirements), a number of alternatives for rearing might be considered. Some of these have already been mentioned but will be noted again:

a. Contract Rearing. The total stock of adult flies in the Bristol laboratory comprised 11,000 flies at the end of 1973 with considerable numbers available for export over and above the needs of the laboratory. It may be possible to contract with the Bristol laboratory to rear flies of the same Tanga strain in such quantity that irradiated pupae can be transported to Tanga for release in the field. This would entail some structural alterations, additional staff and equipment. However, sufficient climatically controlled storage space is available for 40,000 to 50,000 flies. It is conceivable that additional backup could be expected from the Seibersdorf laboratory as well, inasmuch as there already is an ongoing contract with IAEA. Additional backup arrangement might be made with Itard in Paris who is said to have a capacity for rearing, say, 15,000 adults at a time and with Evens in Antwerp, who has a capacity for rearing about the same number as at Seibersdorf. Of course, if subcontracts for rearing were entered into so as to be assured of a sufficient number of male flies for release, additional costs might be expected.

b. Using Easier Reared Tsetse for Release. The matter of strain differences between the *G. morsitans* in Bristol and the *G. morsitans* from

Tanzania may need to be investigated further, and consideration given as to whether the Bristol colony has native or evolved strain differences because of its long laboratory habituation. It needs to be determined if Rhodesian morsitans will mate with the Tanzanian morsitans and to learn further, if such mating takes place, are there offspring and are the offspring fully fertile. On the basis of Curtis'* experience with mating morsitans from Morogoro, Tanzania and the Rhodesian stock at Bristol, it would be expected that the Tanzanian and Rhodesian morsitans will be fully compatible. In fact, such crosses have been made in both directions in Vienna, both being fertile. At this point little is known about the field behavior of the progeny.

c. Maximizing Fecundity. It has been suggested earlier that initial high rates of fecundity in the life of the adult female present opportunity to build up the rate of pupa production for the colony as a whole. Instead of allowing adult females to live out their lives, they might be allowed to live only through their first 7 pupation cycles (the first 70 days, say, of their lives) when the rate of pupa production is highest, then destroyed. In effect, about 80 percent of the pupa production would be accomplished in about 65 percent of the time. As noted earlier, Jordan has had success with this rearing practice.

d. Improved Feeding Methodology. As membrane feeding becomes perfected, it is said to offer time and labor savings that will increase the capability of a rearing facility in its fly production. Further, experience at Bristol and Seibersdorf indicates that, on the average, individual flies take a blood meal somewhat less than daily; it is probable the number of feedings per week can be reduced to 5 or 6 weekly.

B. DEVELOPMENT OF METHODS FOR PACKAGE AND TRANSPORT OF FLIES

1. Goals: Acceptable Loss Rate

On the assumption that flies will be sterilized by irradiation rather than chemosterilization, an average loss of 4 percent should be expected

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*Curtis, C.F. "Sterility from Crosses between Subspecies of the Tsetse Fly, Glossina morsitans." Acta Tropica, 29 (1972): 250-259.

prior to the processing of material for packaging if irradiation is performed on the day of packaging (Curtis and Langley, 1972)*. During the packaging and transport of flies to their release site, a loss rate of an additional 4 percent should be anticipated provided that the transport environment remains within acceptable limits. Most of the anticipated loss can be attributed to unavoidable physical damage to the flies during transport to the release site (3 percent), while a small amount (1 percent) will occur during placement of the flies in a package. Thus, 8 percent should be the minimal loss rate observed. To permit some variance, the maximal acceptable loss rate probably should not exceed 12 percent.

2. Stage of Growth - Adult or Pupa

Several investigators (Bursell, 1961**, Dame et al. 1969*** and Langley, 1970****) have demonstrated that tsetses which emerge in the laboratory have poor development of the thoracic flight muscles and that these flies perform poorly in the field (Dame and Schmidt, 1970*****). Since subsequent research has not produced laboratory emergence of adult flies with flight musculature equivalent to that in wild tsetses, it is recommended that the pupal stage be used for shipment.******

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*Curtis, C.F. and Langley, P.A. "Use of Nitrogen and Chilling in the Production of Radiation-Induced Sterility in the Tsetse Fly, Glossina morsitans" Entomol. Exp. Appl., 15 (1972) 360-376.

**Bursell, E. "Post-Teneral Development of the Thoracic Musculature in Tsetse Flies," Proc. R. ento. Soc., Lond., A 36 (1961) 69.

***Dame, D.A., Birkenmeer, D.R., and Bursell, E. "Development of the Thoracic Muscle and Flight Behavior of Glossina morsitans orientalis Vanderplant," Bull. ent. Res. 59 (1969): 345.

****Langley, P.A., "Post-Teneral Development of Thoracic Flight Musculature in the Tsetse Flies Glossina morsitans and Glossina austeni. Entomol. Exp. Appl., 13 (1970)" 133-140.

*****Dame, D.A. and Schmidt, "The Sterile Male Technique Against Tsetse Flies, Glossina spp." Bull. Entomol. Soc. Amer. 16 (1970): 24-30.

******Bursell et al. (1972) have shown that thoracic muscle growth in laboratory-reared G. morsitans can be accelerated to reach normal levels by inducing flight immediately after the first blood meal. It would seem that this manipulation would not be practical for mass release of sterile males for several reasons.

Two additional factors in favor of the shipment of pupae are that they are less susceptible to injury during shipment than adults, and they occupy less space in a package than would adult flies.

3. Locale (Environmental) Constraints on Viability of Flies

Both the adult and pupal stages can tolerate fairly great extremes of temperature and humidity for periods of up to 6 hours. Flies should survive over a range of 10-30°C. and a humidity range of 20-80% relative humidity; releases may be made without adverse mortality. Exposure to temperature above 40°C., even for a few minutes is known to have an adverse effect on *Glossina*. For transporting tsetse flies to a release site, a suitable temperature range might be 10-15°C., with a relative humidity of approximately 50%. In general, flies which have been immobilized by low temperature transport better than flies which are carried at their optimal rearing temperature of 24°C.

4. Packaging Methods for Optimum Life

Procedures have been developed by Kernaghan and Nash (1964)* for shipping adult and pupal tsetse flies in containers made of 1.5-in. expanded polystyrene foam approximately 7 in. square by 4.5 in. high. The container has a central volume of 500 ml. and will hold up to 400 pupae without causing adverse mortality for 4 days. To prevent damage during transit, the pupae are interspersed among nylon shavings about 1 mm. wide and 0.13 mm. thick. Humidity is kept high by pieces of moistened plastic foam placed at the top and bottom of the box. The entire box weighs 3.25 oz., or 92 gm. Such containers have been used for the shipment of pupae from Zanzibar to England without excessive mortality. Modifications of this package can be made for the Tanzania project.

If refrigerated containers are available during the transport of flies to a release site, then smaller containers can be used which might be suspended in sacks in a portable refrigerator. Depending on the season of the year and the release site selected, it may be possible to use containers of simpler construction such as pint-size ice cream cartons with a thin foam inner layer than can serve both as an insulator and a source of moisture to maintain a suitable humidity.

5. Duration and Maintenance of Transport Environment (Atmosphere, Temperature, Humidity, Etc.)

Many of the details concerning this item are related to the amount of time which will elapse between the packaging of the flies and their release in the control site. The use of refrigerated or air-conditioned vehicles to carry the packaged flies from the rearing laboratory to the field can greatly facilitate this operation. If these are not available, well-insulated picnic-type chests or large, chilled vacuum flasks can be used to transport chilled pupae or adults to the field. Temperature may be kept at a low level by the addition to this large storage container of plastic bags or cannisters filled with ice. Humidity will be maintained at an adequate level by the inner insulation of the smaller containers holding tsetse flies or pupae. With this type of system, it should be possible to maintain a temperature of 10-15°C. for periods up to 8 hours or longer, depending upon ambient temperature.

C. RELEASE METHODOLOGY

1. Mode - Aerial or Ground

There is little information available concerning the aerial release of medically important insects for dispersal studies or control procedures. Approximately 15 years ago, studies were made on the packaging and dispersal of *Aedes aegypti* (the yellow fever mosquito) by a group at Fort Dietrick. This material was never published and is probably still classified. In order to develop a procedure for aerial release of tsetse flies, a number of details require considerable research, i.e., development of a suitable package with adequate insulation; determination of whether the tsetse container could fall free or require a parachute to slow its passage; should the package open upon impact or have a time-release mechanism; and with what degree of accuracy could a tsetse package be expected to reach a given target area? The economics of developing such a methodology for release may be beyond the scope of the present study. It may be possible that the technology of the release of the Mediterranean fruit fly could be adopted to this project.

Although ground release of flies would be more time-consuming than an aerial release and consequently use greater number of man hours per

unit number of flies released, existing technology could be used for this method with a minimal amount of further research required. Furthermore, flies would definitely be dispersed from pre-selected points. This last point is rather important as the flies should be released in a microhabitat which is not fully exposed to climatic extremes such as the mid-day sun or in an area where a container of specimens might be destroyed by cattle or predators.

2. Time of Day

The optimal time for dispersal of sterile males should be early in the morning to avoid the hot mid-day sun which might produce excessive mortality among teneral (newly emerged) flies. If pupae are released, removal from a chilled container should allow emergence to occur 3 hours after removal from the container (Curtis and Langley, 1972)*. Thus, flies which were placed in the field as irradiated pupae between 5 and 8 a.m. should almost entirely emerge from 8-11 a.m., a time when rising temperatures should stimulate fly emergence. Flight should commence shortly thereafter and the flies will seek a sheltered resting site in the shade. It is expected that the first blood meal will be taken 24-48 hours after emergence.

3. Frequency and Rate (as related to level of indigenous fly population)

Most sterile male programs involving insects other than Glossina release a number of sterile males approximately 10 times the size of the normal male population. In the instance of Glossina, Dame and Schmidt (1970) have suggested that 9 times the average normal male population be released as sterile males over the first 3 months (these are released at monthly intervals in groups representing 1/3 of the total), 4 1/2 times the wild male population from months 4 to 6, with a 50 percent decrease each quarter. By the end of the year, the population should be only 2 percent of the initial number. If this concept is followed, it may be readily estimated that very great numbers of flies will be required for an area such as the Mzeri Hill Ranch which contains 162 square miles. If tsetse densities are relatively low, on the order of 200 flies per square mile, a scheduled program of release of sterile males might be as follows:

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*Op. cit.

Natural density at start (males) per square mile x 160	Period (months)	Number of sterile males released each month
100 x 160 = 16,000	0 - 3	144,000 (48,000/month)
	4 - 6	72,000 (24,000/month)
	7 - 9	36,000 (12,000/month)
	10 - 12	18,000 (6,000/month)

Since this is predicated upon a very high production of sterile males in the laboratory colonies, smaller pilot studies should first be conducted, involving only several square miles. Initially, releases might be made at a level of 5 times the normal fly population, and subdivided into 2-3 releases per month. Using the value of 200 flies per mile, in a 10-square mile plot, 2000 flies would be expected, or about 1000 males. If 6 releases are made over the 3-month initial period, then only 1000 x 9/6, or about 1500 males would have to be released every 2 weeks. With a mortality factor of 12 percent estimated previously, about 1700 males should be released. If the population is in the range of 500 per square mile, then 4200 sterile males would be necessary. This model is based upon a uniform population throughout the year. The natural situation would be more complex and computer simulation studies might be required to predict the actual number to be released.

Assuming there is a limit to the numbers of sterile males available for release, the problem might be worked in reverse. If 10,000 males were available for release, then for the first 3 months, a natural density of 42.5 flies per square mile would be required, and if the whole 162 square miles of the test site were treated. It follows of course that the natural population level needs to be reduced naturally or artificially to low levels before the release program is begun.

4. Stage of Growth - Pupa or Adult

It is known that laboratory-bred flies which emerge in the laboratory, have poorly developed flight muscles. As mentioned, this may be overcome by forcing the males to fly immediately after taking an initial blood meal. This additional step would greatly increase the cost of producing sterile adult male flies in the laboratory. With this additional conditioning

it would still have to be determined whether these flies could disperse normally in the field. An advantage to releasing such flies in the field would be that they would no longer be teneral, would have a greater chance for survival and would be closer to an age where they could actively pursue female flies for mating. On the negative side would be the higher mortality which usually occurs in the transport of adult flies as compared to the transport of mature pupae. In addition, smaller numbers of adults can be packaged per unit volume than pupae.

The ease of transporting pupae and the knowledge that adults which emerge from laboratory-reared pupae have normal flight capabilities is advantageous to a pupal release. Disadvantages of pupal releases are possible removal by predatory animals, dessication from excessive temperature, and inability to find a host for an initial blood meal.

With our present understanding of the bionomics of sterile tsetse releases, it would be advantageous to release the pupal stage.

5. Number of Host Animals Required for Maintenance of Fly Population in a Free Test Environment

On any of the cattle ranches which might be involved in the release program, there is a host population adequate to support the released sterile males. An important consideration is the normal pattern of distribution of cattle on a ranch in relation to the location of the planned sites for release of tsetses. Since it is known on the basis of mark and recapture experiments (Harley, 1960)*, that the majority of released flies (probably over 90 percent) never travel more than 3000 yards, it is imperative that the cattle or wild animal hosts are within easy flight distance of a release site. If this is not the usual situation, cattle may have to be tethered in this area to provide a source of blood for newly emerged flies.

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*Harley, J.M.B., "A Review of Entomological Research Carried out by the East African Trypanasomiasis Research Organization," Report of the 7th Commonwealth Entomological Conference, 1960. London, Commonwealth Institute of Entomology, p. 246.

6. Relation Between Pre-Release Insecticidal Control and Viability of Released Flies, and of Indigenous Flies

There apparently is no information available on the effects of sub-lethal doses of residual insecticide upon the behavior and viability of tsetse flies in their natural environment. However, from studies on accidental insecticide contamination of laboratory colonies of Glossina with trace amounts of dieldrin and dichlorvos (Shell vapona strip), it has been shown that the reproductive potential of females drops to such a low level that closed colonies cannot be maintained. There is also a 50 percent reduction in the life expectancy of both sexes and an inhibition of the feeding response. From this evidence, it is suspected that the use of almost any residual insecticide in the field would exhibit a similar effect both on released and indigenous flies. There is some evidence which indicates that older females are more tolerant to organic insecticides than younger flies which should be maintained within the population structure for a sterile male control program.

The duration of the above residual activity would be related to the type of insecticide used, method of application and season of the year. For any residual insecticide applied, a minimal time before any flies should be released would be one week from the date of initial application. If a non-persistent pesticide is used, such as the synthetic pyrethroid, resmethrin (SBP-1382), tsetses could probably be released 24 hours after application (personal communication, G.K. Shumaker, S.B. Penick & Co.)

7. Environment or Climatic Relationship with Viability of Released Flies

It is expected that flies which are released should show a normal pattern of survival within the temperature range of 18-30°C. Flies which emerge from laboratory-reared pupae and are immediately exposed to very low humidity and high temperature (32°C. or higher) might have poor wing development and consequently would perish. Therefore, releases should be made on days which do not exhibit extremes in the range of temperature, excessive wind at the ground level, and rainfall which might splatter against emerging flies.

D. FLY POPULATION DYNAMICS OF PROBABLE TEST SITE OVER A TYPICAL YEAR

It has been reasoned earlier that control and eradication over the probable test site is likely to depend upon combinations of control measures of which sterile male release is the last step in the process, principally because use of sterile male release would be prohibitive logistically and cost-wise when the native tsetse population was high. Dr. Lindquist at IAEA believes that sterile male should be released until the wild tsetse population has been reduced to a small fraction of its original size. It is probable that this will be done at the Mzeri Hill test site using 2 to 3 successive applications of non-persistent insecticide about 3 weeks apart to kill adult flies. The population dynamics of the area needs to be known to some degree before insecticide treatments, and to a high level of detail after insecticidal treatments--but before release. While the insecticide may have reduced the number of adult flies, there are likely to be as many pupae that will emerge over the next 25 to 60 days as there were adult flies initially. The discussion that follows is concerned mostly with population dynamics of the area during and after insecticidal treatment.

1. Definition of Fly Counting Methodology

The standard procedure for sampling tsetse populations is based upon the fly round. The catches are made according to a routine method by trained catchers walking along a fixed route. The path is marked, and is divided into perhaps 12 sectors, corresponding to different types of vegetation. It is usually no more than 5-6 miles in length. The standard fly round is conducted by two men, proceeding at a walking pace. They stop every 80-100 yards and collect all the tsetses which are attracted to themselves, using nets to collect the flies. For certain species, it is necessary to use cattle as bait or a screen of black cloth as an attractant. This is not necessary for G. morsitans. The rounds are usually started early in the morning, when the flies first become active, and rounds are completed about noon time. The results are usually expressed in terms of the number of flies collected per 10,000 yards (5.7 miles), according to different types of vegetation. Each fly round is repeated; the second trip is made 2-3 days after the first starting in reverse order. Full details of

this methodology are described in Mulligan (1970).*

Other methods such as fly trapping and pupal collections provide ancillary data on population trends, but it is the general consensus of tsetse field workers that the fly round provides the most useful data indicative of population trends. When this procedure is associated with mark and recapture studies, further extrapolations may be made on actual population size.

2. Mapping of Site to Show Population Concentration

The data from series of flies rounds can be analyzed, and mean values placed on transparent overlays taped to topographic maps of the site. Various forms of cross-hatching or different colors can be used to indicate areas of similar populations. Grease pencils or "magic markers" may be used on the overlays. These can then be photographed or copied for the use of field teams. The data for each month can be thus analyzed and any consecutive months which show fairly similar population trends should be combined on a single overlay. If more than a single tsetse species is mapped, standard symbols are available for indicating the species involved.

3. Mapping of Fly Host Population Concentrations

Data from the census of cattle and any other large mammal populations on the site can be mapped in a manner similar to that used for the tsetse flies. The respective overlays for tsetse and host populations can then be superimposed upon one another to readily show coinciding distribution patterns.

4. Mapping of Topographic and Vegetation Differences

Adequate topographic maps of the ranch may be available from various governmental agencies or map services. In the event that these are not available, it might be advantageous to ask the Tanzanian government for assistance in mapping. The general pattern of vegetation can best be shown

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*Mulligan, H.W. (Ed.), "The African Trypanosomiases" New York, Wiley, 1970.

by a series of aerial photographs, one set photographed during the wet season and the other made during the dry season. These have the added advantage of showing changes in any bodies of water which may occur on the study site.

5. Relation of Fly and Host Population to Topographic Features and Vegetation Concentrations

The behavior of G. morsitans in respect to vegetation pattern varies so much from area to area that it is impossible to provide much specific information directly relating to the selected study site. There are certain generalizations which apply to G. morsitans in any area--trees and shrubs provide resting sites, shelter from adverse climates and predators, as well as vantage points from which potential hosts may be observed. In addition, the pupae may be found in the soil near the base of suitably sheltered trees. Detailed ecologic study by the resident entomologist should provide the necessary data.

6. Survey of Predator Concentrations in Test Site

The role of predators in the natural control of tsetse flies is poorly known. Virtually all the available information is descriptive in nature and it is not possible to determine what portion of a tsetse population is subject to predation. The known predators of adult tsetse flies include the Asilidae (robber flies), various spiders, birds, and dragonflies. There is speculation that elephant shrews, guinea fowl, and francolins may eat tsetse pupae because they have been observed burrowing and scratching in tsetse breeding areas. Careful observation of the study sites may reveal predators, but it is suspected that this type of survey will not be very productive. There should be special attention paid to pupae release procedures, however, to determine that predators are not finding released "caches" of pupae and destroying them before the flies eclose.

7. Relation of Onset of Barrier Condition to Fly/Host/Predator Concentrations

Unless the barrier contains fencing and/or destruction of the larger game mammals, changes in the concentration of hosts is not likely. As mentioned above, the predator interactions are largely unknown, so a review of barrier effects would be of little value now. In large savannah

tracts, the creation of a barrier should not appreciably change fly concentrations unless the barrier restricts or excludes hosts of the fly. Sampling should, however, include barriers to some extent.

E. CONSTRUCTION AND EVALUATION OF BARRIERS

It has been presumed in the study plan for the sterile male release test that it will be necessary to somehow isolate a test site to make certain that there is no unknown addition or diminution of flies in the test area except by natural means, or increased by released flies. Since one of the objectives of the project is to suppress all tsetse flies of a species within the area, isolation of the area becomes quite critical. Were it not necessary to completely eradicate the flies in the area but simply to conduct a sound experimental study demonstrating the utility of the sterile male release method, no barrier might be needed at all. Releases might be made in a prescribed central area and then response measurements (counts) could be made from the center of the release area outward to distances 2 to 3 miles in all directions beyond the release area. But if an isolated area is required, the criteria for such an area need to be discussed.

1. Criteria for a Barrier

a. A barrier around such an area should be of such a nature or of such extent and dimensions that it will not permit the movement of tsetse flies across the barrier. This means that a barrier may be one in which the environment is such that it prohibits movement across it, is a disattractant to the fly, or has been so treated chemically that the tsetse is diverted from crossing or entering.

b. Other things being equal, the barrier should not permit movement of host animals across it; in other words it should discourage or at least not encourage movement of hosts across the barrier. This is a requirement which will be hard to enforce for wild game as it is less likely to observe the barrier in this fashion. The livestock ranging in the area would be more likely to observe the barrier, especially if there is less forage in the barrier area, e.g., where there has been sheer clearing or there is a cultivated sisal field.

c. A barrier should be ecologically acceptable. That is, if a barrier were 1 or 2 miles in width it would be unwise, say, to apply chemicals that would kill all vegetation in the area or to so change the character of an area that it could not later return to its natural state.

d. The barrier must be politically acceptable. That is, if the Tanzanian government, say, were firmly opposed to sheer clearing of an area, it would then be unwise to push for something like this as a barrier condition.

e. The barrier must be within cost limits. It was noted earlier that the budget cost for preparation of the necessary barrier to be created by sheer clearing was estimated to be about $55,000. The probable test site would require for most complete isolation a barrier 2 miles wide, requiring the clearing of about 46 square miles. It also was noted earlier that from 4.2 to 9.8 acres (median of 7.0) could be sheer-cleared per hour by two D8 Caterpillar tractors. At the median rate some 525 8-hour days of work would be required to clear the 46 square miles--which might not be within cost or time limits.*

2. Natural Barriers

When possible it is best to use natural barriers that are abrupt changes in terrain, such as mountains, bodies of water, wide streams, abrupt changes in the vegetation, and so on. In the case of the Mzeri Hill Ranch area, these natural barriers are not too evident. One boundary of the ranch area is formed by a sisal plantation; neither hosts nor flies are likely to be found very far into the sisal fields. The rivers on the north side of the ranch probably are of sufficient width to themselves serve as a barrier to the tsetse.

3. Artificial Barriers

An artificial barrier will need to be created for about 23 miles of the perimeter of the test area. As already noted, it is planned to sheer

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*There is an indication that the vegetation in the test site is not as dense as in Uganda, where these estimates were made. Consequently, 25-50 percent more acreage may be cleared daily in Tanzania. However, due to increases in cost of gasoline and labor, the actual costs may be similar today.

clear a 2-mile swath around this perimeter. It is intended also that an application of a persistent insecticide be made across the cleared barrier to further insure that no flies cross or enter the area. Other things being equal, it would also be well to choose an insecticide that has a repellent character--to simply keep the flies in, and out of the area. It is likely that the test site itself will be sprayed with a non-persistent insecticide several times before the release program begins so as to reduce the existing tsetse population to a level that is manageable by the release of sterile males. The non-persistent insecticide that would be used, however, would lose its lethal qualities very quickly after being applied.

The persistent insecticide should persist for some time, although additional applications may be necessary to assure that a barrier is being maintained. The possibility of using insecticides alone to create a barrier, in the absence of sheer clearing, may need to be considered if costs and availability of personnel and equipment preclude sheer clearing. Alternatively, it may be possible to clear a 1-mile wide swath, and extend the additional mile by insecticides alone.

Another consideration in developing a barrier is the cost and effort required to maintain it. Since the tsetse is attracted to shade of some sort, new high vegetation may need to be cleared from the barrier zone. How frequently reclearing would be necessary is not known, but probably no oftener than annually. The trade-offs in cost and effectiveness as barriers, respecting cleared versus insecticide, as well as maintenance to continue barrier effectiveness need to be considered in barrier selection and design.

III. PROBABLE TEST SITE

A. INSPECTION OF TEST SITE

The probable test site for the release program is the Mzeri Hill Ranch, a nationalized cattle ranch of 162 square miles over which some 14,000 head of livestock graze. The ranch area is about 65 air miles or 80 road miles west of Tanga on terrain that may be described as a bush or savannah area, with grasslands and grasses growing well in the rainy season and low shrubs and trees remaining during the rest of the year. It is an area that is populated largely by the G. morsitans group of tsetse flies as shown in the accompanying map from Ford. (Fig. 4).

The specific location of the Mzeri Hill Ranch is shown in Fig. 5 As is seen, it lies about midway between Mombo and Handini and is a 3-hour drive from Tanga over fairly rough roads.

The ranch area is divided up into 38 "paddocks" as shown in Fig. 6. The paddocks average 2700 acres, and give a kind of order to the land area. The lines demarking the paddocks are perhaps 100-feet across, having been cleared to some extent; it generally is possible to drive a 4-wheel vehicle down the paddock boundary lines. The fact that these paddock boundary lines have been cleared may make for greater ease in implementing the release program and the fly counting required for the study.

The elevation of the ranch area is 1500 to 2000 feet above sea level; the terrain is gently rolling. It would not appear that 14,000 head of cattle would over-graze the area as this presents on the average one animal for every 7 acres. When observed early in September, however, some portions of the area appeared to be over-grazed and some erosion noted.

Some temporary buildings have been erected by the Tanga project to house equipment and personnel while they are in the field. Water has been run to the site of this field camp so that some amenities are available. It is intended that more buildings will be erected within the next year. A sisal plantation borders the north side of the Mzeri Hill area so it will

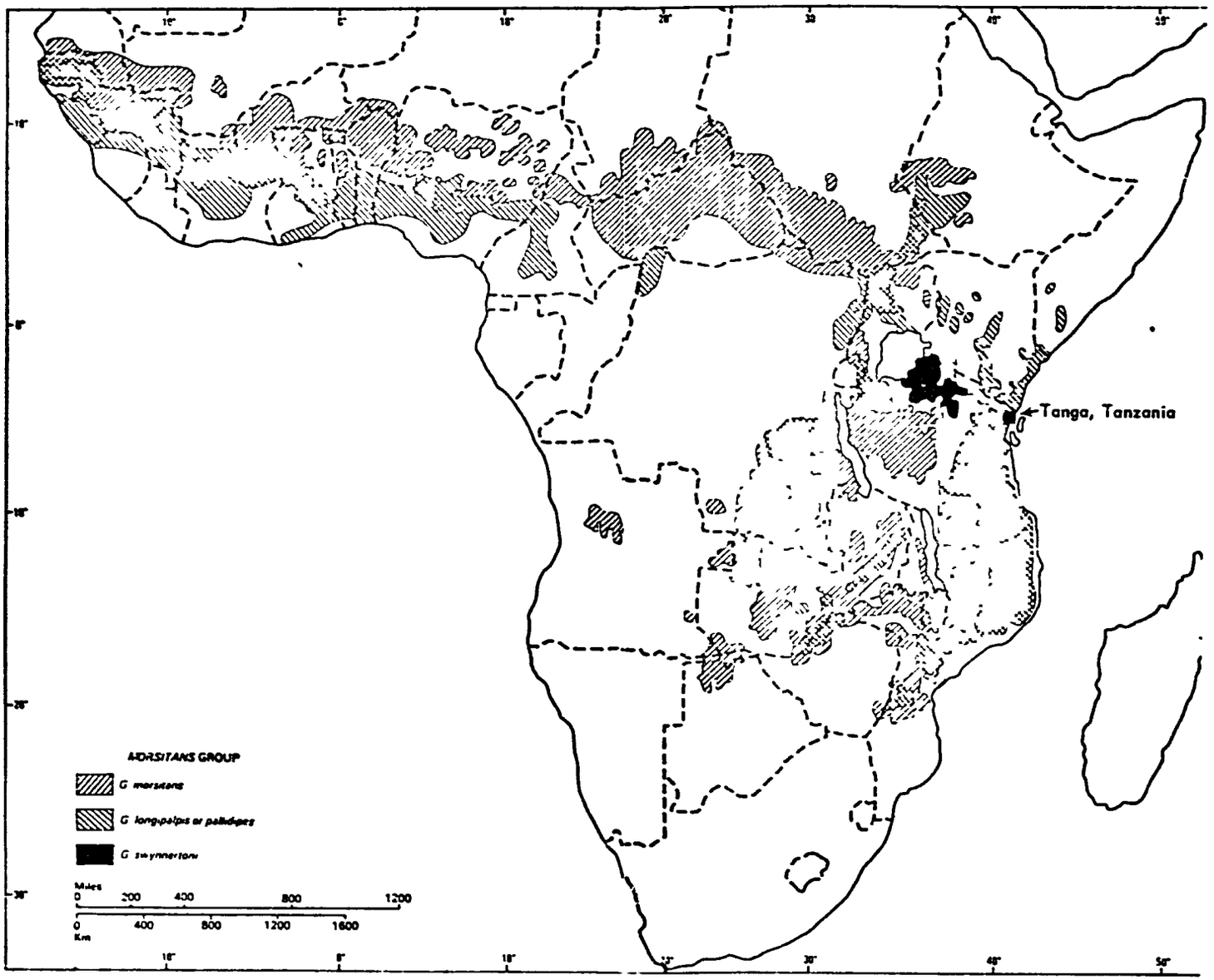

Fig. 4—Distribution of *G. morsitans*, *G longipalpis* (West of 25°E.) and *G. pallidipes* (East of 25°E.), and *G. swynnertoni*
Source. From Map 3.3 as found in Ford, John, "The Role of the Trypanosomiases in African Ecology," Oxford. Clarendon Press, 1971.

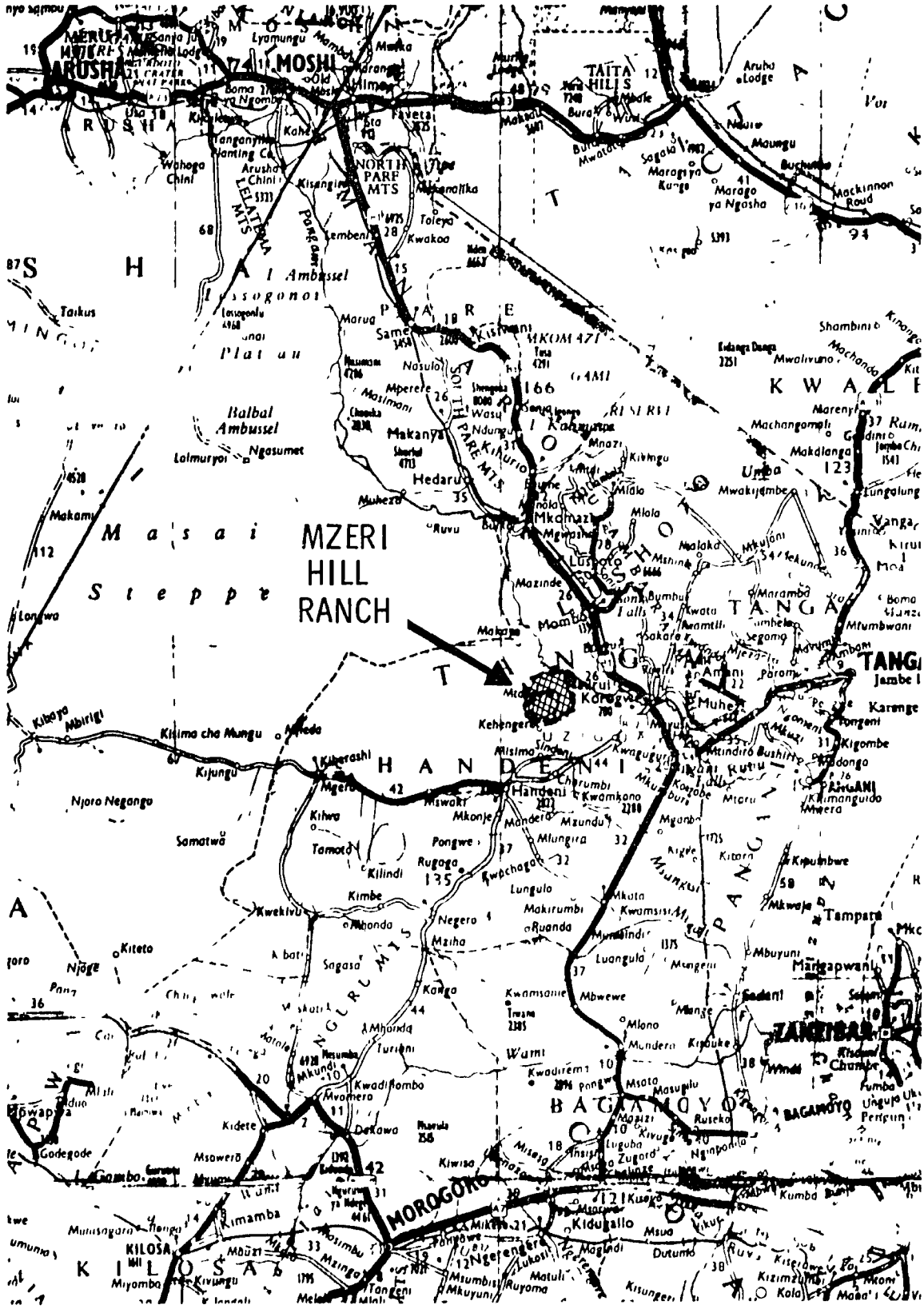

Fig. 5—Location of Mzeri Hill Ranch in Tanzania

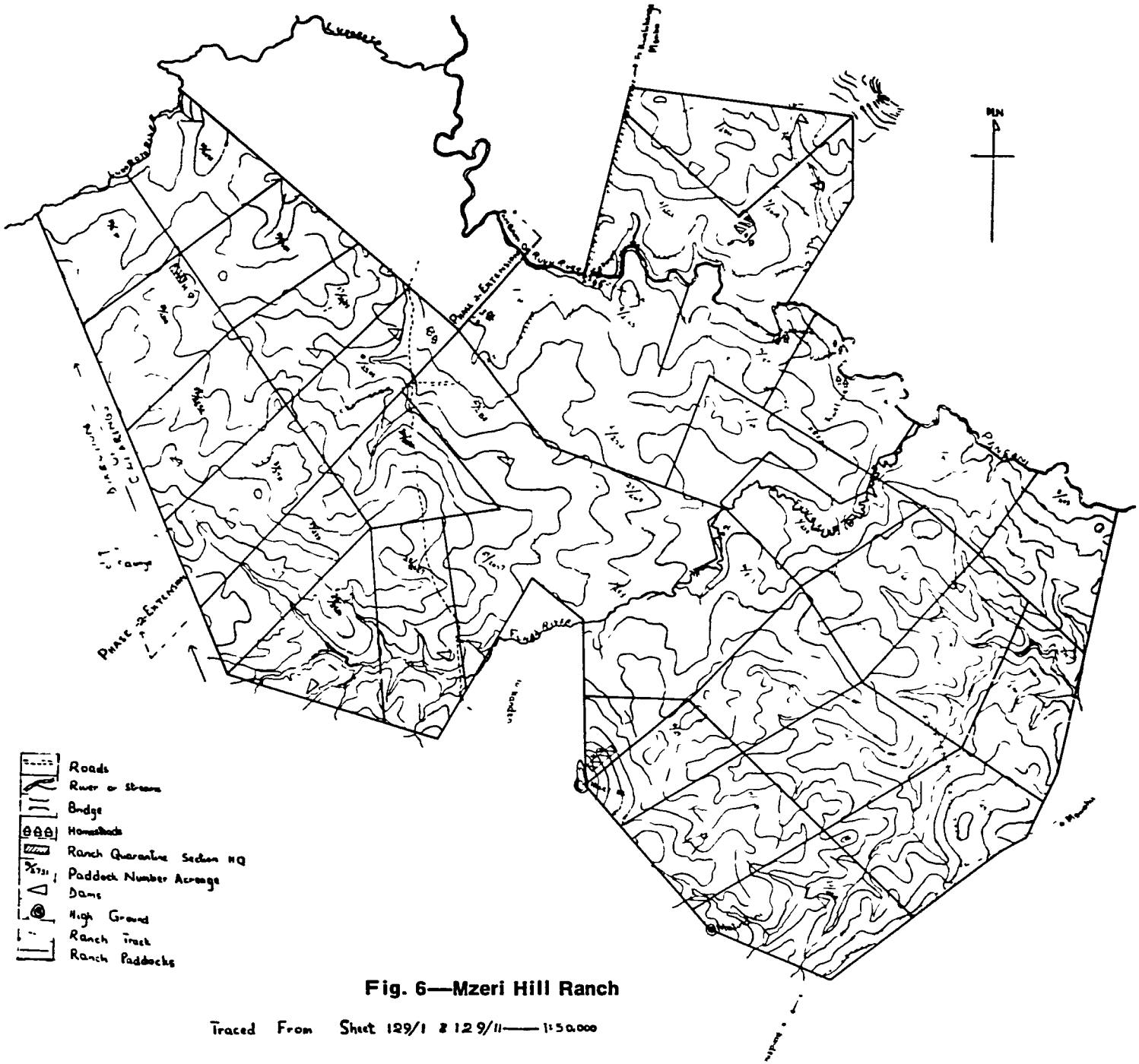

Fig. 6—Mzeri Hill Ranch

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form an agricultural boundary or barrier along that side. This means that the barrier needs to be constructed around the southern, eastern, and western edges of the Mzeri Hill Ranch. It is not known whether the barrier will be constructed on any of the ranch property or not but it is suspected that all sheer clearing that is done will be on non-ranch property.

While the area was visited at a time of year when there should not be many tsetse flies about, and also was an area in which some clearing had been done and a relative infrequency of flies would be expected, quite a number seemed to be attracted to the vehicle. Some were observed when walking outside, not in the vehicle. The Tanga entomologist who has spent most time at the test site area believes that while morsitans is the predominant species in the area that there are occasional austeni and pallidipes picked up as well. From an experimental standpoint, the presence of species not being released will serve as a control by which to further measure the effectiveness of the sterile male release method. At any rate, care must be taken when fly counts are made before, during, and after the experiment to make certain that there is proper identification of the species of tsetse collected.

B. PRELIMINARY FIELD TRIAL DESIGN

1. Objective

The objective of the field test is to establish and test a methodology for the control and suppression of G. morsitans in a test area isolated by barriers to preclude movement of tsetse flies into or out of the test area.

While the morsitans species predominates throughout the test area; it is recognized that other species of flies--pallidipes, brevipalpis, and austeni also may be present in the test area in small numbers. So, while control and suppression of morsitans may be effected by the sterile insect release method, control over these other species must be effected by other methods, principally, control by insecticides.

2. Method

a. Experimental Design. Current planning for the full field test puts the test in FY 78 with some possibility that it be conducted in FY 77.

The full field test then is probably about three years away and many assumptions need to be made--as to the availability of resources (flies, personnel, funds); the objective of the study, as to whether a limited objective (smaller control area, varying control treatments) could be accepted; and other interesting design considerations. These possible constraints suggest prudence to the effect that a number of alternatives and alternative designs be considered.

There may be confusion in the goals of the tsetse project in Tanzania. The confusion is in the sense that the Tanzanian government expects that the Mzeri Hill Ranch will be essentially tsetse-free at the conclusion of the study, and that it will not be difficult to keep it that way. It needs to be recognized that several factors may prevent this from happening. First, as noted earlier, there is more than one species of fly endemic to the Mzeri Hill area, and the sterile male approach contemplated will control only the species released, morsitans. Second, if eradication is the goal, it might be possible to eradicate the fly by combination of means as effectively as the sterile male release method. Third, if some sort of limited objective could be adopted, one might be able to demonstrate the relative effectiveness of the sterile male release method as well as insecticide control. It is strongly suggested that a concept of limited objective(s) be adopted; the gain to scientific research in the area of tsetse control and to the Tanzanian government as well, will probably be highest if this is done.

Constraints upon resources may well determine the objective that one seeks. Assumptions are being made that there will be sufficient excess of adult male flies for release to properly carry out the experiment over the entire 162 square mile area. Resources that may be in short supply are flies, personnel, and equipment. It is assumed in program plans that there will be available out of the Tanga facility some 30,000 adult male flies per month for release purposes. As noted earlier in the report, this many flies may require contracting for fly rearing at sites other than in Tanzania if production does not achieve expected levels. Or it may be decided that indeed this many flies are not required. In the examples

discussed earlier, if flies in the native environment are reduced to a level of, say, 100 females per square mile, than at a minimum, about 81,000 flies would be needed at any one time to flood the entire test area at a rate of 5 sterile flies to 1 wild male, or about 50,000 at the rate of 3 to 1.

In practice, it probably would not be possible nor necessarily desirable to release this many sterile males at one time; the scheduling of release likely will require smaller numbers per day distributed over a longer period. It has been suggested by tsetse experts that a release experiment will have the highest chances of success when the indigenous tsetse population is reduced to as low a number as possible before sterile male release. This is recognized as desirable when eradication of the species is the object. However, in the conduct of an experiment where data are needed to demonstrate the effect of a treatment, it appears undesirable to reduce the wild population to a point where sampling procedure would no longer be effective. It is felt by the authors that initial reduction to any level less than 100 wild males per square mile is the lowest that could be tolerated if one still hopes to collect any significant numbers of flies by any of the sampling methods. The figure of 100 males is highly speculative, (perhaps it should be much higher), as it is not known what proportion of flies in a square mile area would be collected by a fly round passing through the area; the appropriate number should be verified. The issue of the proportion of absolute number of flies per square mile (as determined by mark-release-recapture experiments) that will be collected in a normal fly round needs high priority. This is a matter that needs to be settled before sterile male release on a large scale is begun; an otherwise sound experiment could be jeopardized by too rigorous initial insecticide killing of flies, leaving none to count.

Since there is the possibility that larger numbers of flies might be required than anticipated originally, it might be wise to make certain that there was a back-up supply of male flies regardless of how well the Tanga Project appeared to be producing them. On the other hand, it will be possible by the experimental design chosen to reduce the amount of release area on a logical basis so that the requirements for flies for release

purposes are not as great as supposed.

Another constraint may be the amount of money available for insecticide applications. It is understood that the Tanzanian government will furnish equipment and personnel (as well as insecticides) for insecticide applications to the barriers for killing flies in the pre-release program, and for any experimental control program on the test site which might be included in an alternative research design. The history of Tanzanian support indicates that this commitment might not be dependable, and that some contingency should be developed to assure that there is equipment and insecticide available to conduct the study.

Similarly, the equipment, personnel and time necessary to clear the barrier for the test area needs to be assured. It has been demonstrated earlier, that under a fairly optimistic assumption, a great effort is required for the clearing operation. Again, the Tanzanian government is obligated for the clearing. It may be necessary to cut back in clearing requirements; an alternative design requiring less clearing should be considered and be ready for implementation.

There are a number of possible experimental design can be described conceptually (the nature of the terrain, layout of the ranch area, grazing practices, high concentrations of flies in certain areas, etc. may dictate details of the design); four of these possibilities are diagrammed in Fig. 7.

The first design is full release over the entire ranch area, with a complete sheer-cleared barrier two miles in width. This is the design that has been contemplated, contingent upon availability to produce enough flies and availability fo equipment and personnel to sheer-clear the entire barrier area. In some ways, it is a very clean test of the sterile male release methodology, in that it represents a completely isolated area. In other respects, it is not as attractive, because the only kind of control condition possible is use of flies indigenous to the area that are not of the <u>morsitans</u> species, or selecting an area outside of the barrier zone that is equivalent in character to the Mzeri Hill area and that has similar

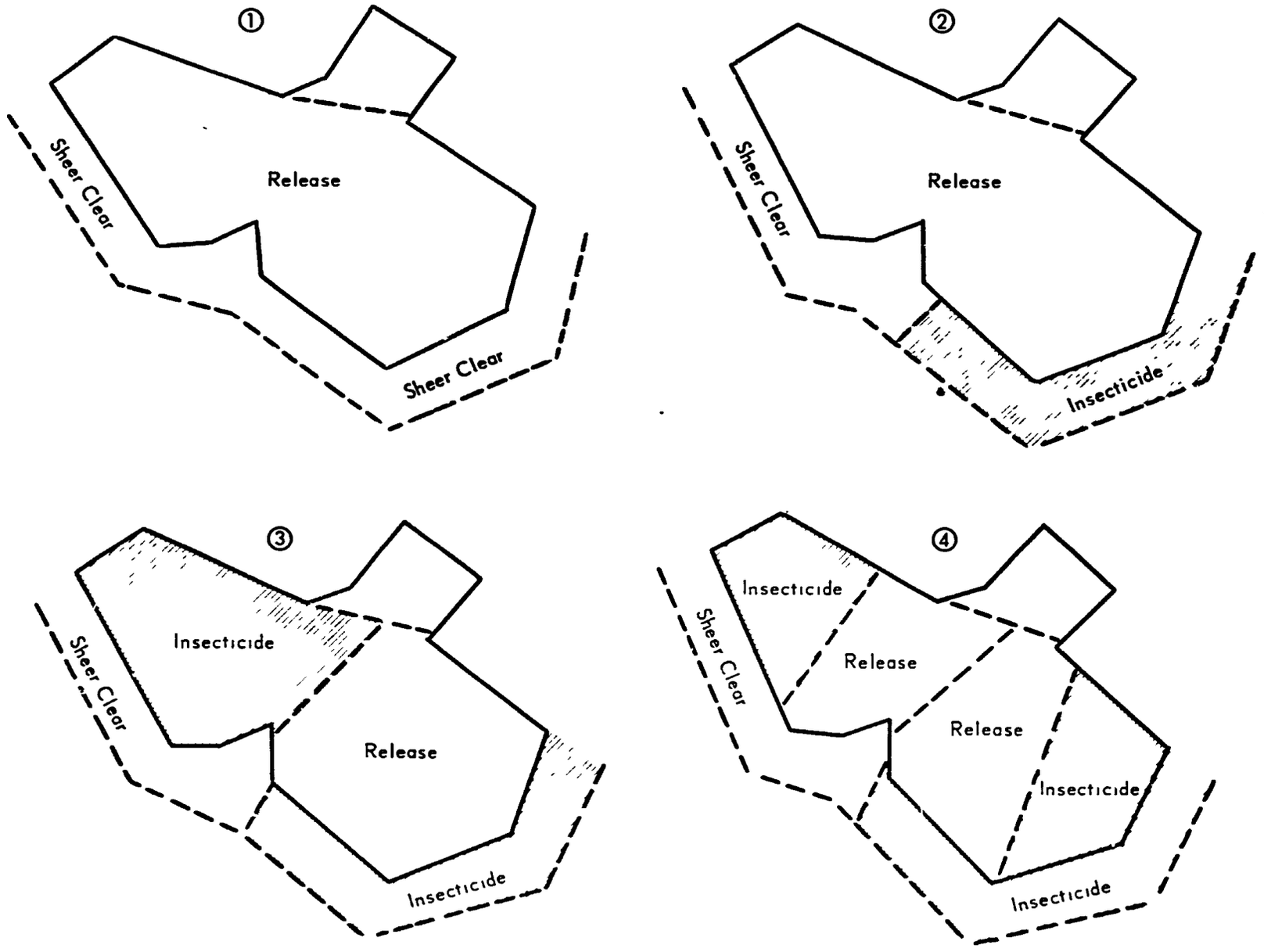

Fig. 7—Concepts for Experimental Designs

concentration of morsitans.

The second design can be described as full release, with two kinds of barrier conditions. Release of flies would be over the entire test area but the barrier is divided into two sections--1/2 sheer-cleared, and 1/2 treated with a persistent insecticide. In this design, it might be assumed that there was a constraint upon the ability to sheer-clear the entire area, so a persistent insecticide is used as another means of isolating the test area. The persistent insecticide that will be used in a case like this would be several treatments with dieldrin or DDT or an insecticide with similar lethality.

The third design is 1/2 full release, 1/2 insecticide for the control treatment in the test area, and 1/2 sheer-clear and 1/2 insecticide as the barrier treatment. In this design, release is in the eastern portion of the range area with an insecticide barrier contiguous to the release area. In the test area where control is being established, a non-persistent insecticide, such as resmethrin or pyrethrin, might be used in the test site area, or a persistent insecticide could be used. Again, the barrier is treated in the same way as described for the design preceding (i.e., 1/2 sheer-clear and 1/2 persistent insecticide). This design, then, also assumes some constraint on the availability to sheer-clear the entire barrier area and, in addition, assumes a constraint on the availability of flies.

The fourth design represents combinations of the same treatments just described for the third above, but makes for greater variation in the contiguity of treatment and barrier. Thus, one ends up here with four different combinations of treatment and barrier. From a statistical standpoint, this design offers the experimenter the most information. The assumptions about constraints here are the same for the previous design.

Numerous other designs are possible. For example, one might have a narrower sheer-clear area for the barrier and have it bounded on one or both sides with a persistent insecticide treatment. Or indeed, one might have a situation where there is no barrier at all--only release from a central point. Measurements of levels of control would then be gradients

of fly density in all directions from the central point of release. This kind of design might not be as satisfying to a customer or client, but it appears that it could be quite effective and be much less costly than one in which extensive sheer-clearing or insecticide treatment to create a barrier were required. Still other possibilities where sheer-clearing might not be required at all are possible; for example, the entire barrier could be created using a persistent insecticide.

In Appendix A, a "strawman" design is developed in which a more or less idealized experimental design is presented. The strawman does not presume to be a recommendation as to the form the Tanga field experiment might take eventually. Whether or not the strawman design is adopted is not important. Its purpose is to explicate details of experimental control thought to be important to a field experiment of this type. The elements of experimental control described are important.

b. Isolation of Test Area. An agricultural barrier of extensive fields of sisal isolate the test area on the north and east. The sisal is an effective barrier inasmuch as there is no food for livestock there and few other wild host animals will find this a suitable habitat. Nor does sisal provide the kind of vegetation the tsetse seeks. The whole northern edge of the Mzeri Hill Ranch also is bounded by streams (sisal fields being on the other side) which improves this as a boundary (although there may be some riverine species of fly that might be attracted to the stream vegetation).

Sheer-clearing is an effective control, so long as the clearing is maintained. It is speculated that additional cutting or clearing must be done at least biennially, if not each year, in order to keep the sheer-cleared area in that state. Sheer-clearing is effective because it removes the habitat that the tsetse prefer; it appears that even though host animals might wander through the cleared area, if the area is extensive enough, the tsetse is not likely to follow the host animal any great distance.

The use of herbicides and plant defoliants can be most effective in establishing a barrier area. These, however, have the disadvantage of tending to

permanently change the ecology of the area and threaten adjacent areas. If sheer-clearing were pursued too vigorously, it could conceivably have the same effect.

Persistent insecticides may be used to create a fly barrier. They are not tsetse-selective, and also will kill other kinds of insect life, some of which might be beneficial. Many of the persistent insecticides leave a residue which may have long-term deleterious effects on man and domestic animals, and threaten the natural ecology of a region.

c. Reduction of Indigenous Tsetse Population to Low Level before Beginning Release Program. It has been noted previously that it is important before release to reduce the indigenous fly population to as low a level as is consistent with sampling methodology, and contingent upon the numbers of male flies available for release and the area over which release is to take place. A remaining population of no less than 100 male flies per square mile has been suggested tentatively earlier in this paper.

In reducing the indigenous population, there is some question as to whether a persistent, or a nonpersistent insecticide should be used in that portion of the test area that subsequently is to be used as a release area. A combination of persistent first, followed by a nonpersistent, is possible. The nonpersistent only, applied two or three times, would seem to be a safer practice, as one would not wish to kill off released males with a toxic residue remaining. Of course, a persistent insecticide would be recommended in the barrier, or just outside of the barrier area. As noted previously, resmethrin or pyrethrin is suggested in the test area, and dieldrin or DDT in the barrier area.

The mode of application is not established. It is probable, however, that aerial application will turn out to be the most efficient, even though it is recognized that tsetses resting in the protection of vegetation might not be affected by an aerial application. It is presumed that the aerial application of insecticide would be conducted by the Tanzanian government, which, it is reported has a light aircraft fitted with a variable jet

nozzle for insecticide applications. The number of applications will depend upon the initial density of flies, the insecticide used, the effectiveness of the application, and the frequency of applications. If the original density is somewhat low, say up to 500 flies per square mile, it may be that only two applications (about 3 weeks apart) will be necessary to reduce the density to a point that the release can be carried out. A sufficient interval must have elapsed after the last insecticide application before sterile males are released or sterile males will of course be killed by the insecticide remaining.*

d. Sterilization Procedures. Procedures for sterilizing the male fly have been developed by the IAEA (along with the Bristol laboratory, and the Paris Institute d'Elevage et de Medicine Veterinaire). Similar facilities for sterilization are being built at Tanga. The Tanga radiation source and methods are being indexed to the IAEA source, so that the same levels of sterilization can be applied.

The sterilization of the male pupae requires 12-15 Krads of gamma radiation in air, or 17-19 Krads in a nitrogen atmosphere, rendering 90-95 percent of the adult flies sterile. Separation of the male from the female pupae is facilitated by the fact that female morsitans eclose about 2 days before the males. By using pupae of known age reared under controlled conditions, one can remove about 90 percent of the females for recycling in the colony before the male pupae are collected for sterilization.** Following sterilization, it is possible to chill the pupa and hold off eclosion for a few hours without any harmful effect to the adult fly. If the pupae are to be transported for any distance or to be chilled for

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*If a synthetic pyrethroid such as resmethrin is used, then sterile male releases may be made within 24 hours after application.

**This means that about 10 percent of the "male" pupae being irradiated actually are female, so in any pupal release, that proportion will be female; it is presumed that the female flies emerging also will be sterile, but they probably will be just as attractive to male flies. Thus, for example, if it were ascertained that there were 200 wild flies per square mile--presumed 100 male and 100 female--and 500 sterilized pupae were released, the release in actuality would be about 450 sterile males and 50 sterile females. It can be seen that this makes for some arithmetic problems that must be taken into account when fly counts are made.

any lengthy period of time, say for as much as 24 hours, it is a question as to when irradiation should take place--before chilling or after the transportation in the chilled environment. At this point in time it is not known if the sterility and tolerance characteristics of the Tanga strain of morsitans radiation levels have yet been determined; of course, this will be done before the mass release experiment.

e. Sampling Methodology. Considerable experience should have been developed prior to the field test in the counting of adult flies and determination of the apparent density of flies within some spatial bounds. This will involve considerable familiarity with methods for making estimates with the use of mark-release-recapture techniques to determine the absolute density in an area, and familiarity with the appropriateness of various kinds of fly catching techniques as they are related to the species, sex, and environment of the flies. At the present time, there are no staff persons at the Tanga facility with sufficient experience in this area. So it is extremely important that staff persons be trained in these techniques or be exposed to persons who are especially knowledgeable in these areas.*

At the risk of some repetition of material already discussed, fly population dynamics will be covered fairly comprehensively in this section.

Although considerable effort has gone into the study of dispersal and population density of Glossina, the techniques and methods for such study are still under development. Use of these methods in a field test and fly population control program may furnish research data regarding these methods and their usefulness in providing information for other large scale tsetse control operations.

For this purpose, it is assumed that estimates of absolute numbers of flies per unit of land area as well as relative concentration and dispersal

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*It is understood that arrangements now have been made for both Dr. Gregory and Mr. Gates of the Tanga Project to spend several weeks with Dr. LeRoux in Kenya to become familiar with tsetse and trypanosome survey methodology.

over time are needed. Since the proposed control method depends on the ratio of males to females and sterile males to indigenous males, the knowledge of these components of the fly population are required. In order to evaluate the results of a control program, information with respect to target species and any others present in the test area will be required not only before a control program is undertaken, but during the program, and for a considerable time (say, 2 years) afterward. Some knowledge of puparia density and location also may be needed if density and numbers of emerging adults should affect the sterile fly release strategies.

The usual techniques* for measuring changes in numbers and composition are as follows: (a) moving catches or fly rounds, (b) standing catches, (c) traps and (d) searches for resting flies. Male tsetse flies are often attracted by movement of vehicles, people and animals through their habitat. These males are mostly sex attracted,** not hunger attracted. Apparently, males make contact with teneral females that may also be attracted to moving objects. Some females are attracted presumably for the purpose of finding food. There is some evidence that females are attracted to moving objects but are more disperse and do not alight.***

(1) Fly rounds. Flies caught on fly rounds generally contain less than 10 percent females and teneral males per 10,000 yards. This is termed the apparent density. Whether such practice gives a more accurate index than the whole catch has not been determined.**** However, indications are that well-fed females are not attracted to (or not caught by) fly rounds. Females attracted to fly-round parties have been found to be very hungry. Jordan*** cites evidence that man is an unwelcome host for morsitans and may actually repel the female tsetse. Hence, the catch of males under

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*Mulligan, H.W., op cit.

**Glasgow, J.P., op cit.

***Jordan, A.M., "Recent Developments in the Ecology and Methods of Control of Tsetse Flies (Glossina spp.) (Dipt., Glossinidae) - A Review," Bull. ent. Res. 63, 1974.

****Mulligan, H.W., op cit.

fly-round conditions as conducted historically may serve only as an index of the whole population.

The difficulty is that such an index depends upon the sex ratio being known. This is not the case. Females are thought to live longer than males and the ratio or difference in life span is probably not a constant. It could vary with a number of conditions, such as the presence of predators. At most with fly rounds of the traditional type, one can achieve an estimate of relative density but not the absolute numbers in the population.

(2) Mark-release-recapture. Absolute numbers have been estimated by the method of marking, release and recapture.* Assuming birth rate, death rate and immigration and emigration constant, the following proportions are expected of random samples (a condition usually somewhat in doubt):

$$\frac{\text{Total number of flies in the area (to be estimated)}}{\text{Total number marked flies released (known)}} = \frac{\text{Unmarked flies in sample (known)}}{\text{Marked flies captured in the same sample (known)}}$$

Ingenious methods of marking have been developed not only to facilitate following the flies' movements but also to record coded information regarding point and time of release, hunger condition of the fly, etc. This offers numerous possibilities even when some of the above conditions or assumptions are not met since flies may be caught and recaught a number of times (see Buxton, pp. 446-453 for calculations). This method runs a risk of bias because flies captured more than once may differ from the typical or representative. However, once absolute numbers are determined, the practice has been to estimate the proportion of the population which is caught on a fly round (availability). Conversion factors can then be determined to convert the catch on a standard length of fly round to populations of non-teneral males and females per square mile. Ten thousand yards is the usual length of the fly round specified, but the number of stops, type of party and

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*Buxton, P.A. "The Natural History of Tsetse Flies," London; H.K. Lewis & Company, 1955.

length of stops, etc. is not standard among researchers. Presumably these conditions must be carefully specified and held to in any surveys where the results from one time and place to the next must be comparable.

The moving catch or fly round capture method has an advantage when changes in population size are under investigation. This method usually produces more flies upon which to base estimates. There is considerable question with respect to its effectiveness as an indicator of spatial distribution.

(3) Stationary bait animal. Sex ratios and spatial distribution have been estimated by use of a stationary bait animal (usually an ox) moved into selected positions at a time of minimum fly activity. Sex of flies that come to feed is recorded. The sex ratio so estimated is adjusted by the difference in feeding interval. The latter is estimated by observation of the time between feeds by the same flies. For this purpose, flies have to be marked as they engorge. Evidence so far obtained supports the view that there is no significant concentration of morsitans in a particular vegetation during any time of the year.

(4) Traps. Although site effects and day to day variability are not obtained with trap catches, this method may have advantages when investigating dispersal of flies into open areas. Evidence indicates that traps in this situation do not attract flies from a distance over 100 yards. Also, their continuous operation is an advantage with sparse populations.

Trap system results have peculiarities. Why tsetse enter traps is not fully understood. Further, the results of trapping do not correlate well with those of the fly round or the bait ox. Traps tend to catch more females. This supports the hypothesis that morsitans females are actually repelled by man since one or more men are usually a part of the fly round.

The casually unexplained differences in results from the various techniques of sampling indicate that the techniques are imperfect. While this is no reason to dispair, it means that the users must be knowledgeable both with respect to the techniques, the object fly population and behavior and also the uses to which the estimates will be put.

(5) Catches of resting flies. The size of the catch of resting tsetse is unusually dependent on the skill and diligence of the sample collector. The flies have protective coloration. For this reason, this method cannot be relied upon to estimate variation of numbers of flies in the population. It is also difficult to avoid counting flies that come to investigate the commotion created by the sample collector. This problem is serious enough to bias the results of this method. However, when information regarding the whereabouts of the inactive flies is needed, this is the only currently available way of finding it.

(6) Counting puparia. Although it is possible to locate puparia of Glossina, this does not appear to be useful as an accurate indicator of the population size and density. Usually, the numbers of puparia which can be located in the field do not represent a population sufficient to maintain the numbers known to exist in the population. Various reasons for this include the likely possibility of large sampling error due to the searching team's failure to locate puparia. On the other hand, when knowledge of location of emerging or possibility of emerging fly densities is needed, puparia hunting and sampling may be useful.

(7) Comparison of procedures. A comparison of fly counting procedures shows that the results are not entirely consistent. Traditional fly rounds and other moving catches count more males than females. Standing catches do better in this respect but the presence of man as a deterrent to the flies approach has not been fully investigated. On the other hand, the stationary bait animal does not attract as many flies as the moving catch. In low density populations this may be an important factor. Traps have the advantage of continuous operation and hence may help with a low density population problem when even the presence of tsetse may be useful information. They also have some advantage at points of migration across barriers or into grassland areas. Sampling resting flies does not give a good indication of numbers due to the skill required by the counter and the bias of counting commotion created by attracted flies that show up.

It may be concluded that there is no one technique which serves all purposes. Perhaps the fly round can be used as the basic tool to estimate population densities and density variation over time and space. Modifications of the technique such as use of vertical electric grid of fine wires around or following the "bait"* might be used to help calibrate the ratio of females and males to the "apparent density" figures of the more traditional methods. The other methods may, however, be used from time to time to serve as a check on the fly rounds and to supplement their information. Traps may have a useful place in checking migration. The capture-release-recapture technique in conjunction with techniques of marking is probably the only way of estimating the absolute number of flies in the test area.

(8) Concluding notes on methods. A number of procedures need to be worked out on the test site finally chosen. Fly rounds can be run over prescribed paths or the paths can be randomized. Which method is used depends partly upon the practicality of randomization given the terrain, vegetation and logistics. If permanent rounds are used, a randomization technique for stops and perhaps lengths of stops should be considered. Further, the fly round(s) may be "stratified." That is, either separate rounds or stops classified by such characteristics as area, vegetation (grass, thicket), proximity of hosts, time of day, season, etc., may be used. Proper recording of data and sample procedures (stops, etc.) within these strata will permit later analysis of the relation of these factors to population dynamics. It may be desirable to duplicate certain elements of the sampling pattern before, during, and after the control program. Many of these decisions will have to be made after the test area is mapped. Some will depend on the nature of the exact control program. They can, however, give information relative to male sterile release point strategy and/or insecticide application strategy.

f. Release Methodology. A number of points pertaining to the release of flies have already been discussed. It appears that a ratio of

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*Jordan, A.M. (1974), op cit.

from 3 to 1, up to 9 to 1, of released sterile male flies over wild males will be necessary. The greater the dominating ratio possible, the greater the likelihood of control being achieved.

How frequently flies should be released at a particular release point is debatable, probably no more often than weekly and no less often than monthly. It is probable that the release procedure will be from containers placed in an appropriate place on the ground, rather than from the air although the latter possibility should not be completely ignored. However, if release is from the ground, and release is generally in areas where there are probable concentrations of flies, it is likely that the release of flies will be a continuing process and go on daily over the test area. That is, unless a large staff is used for this purpose, it will be difficult to get to all release points very frequently. Of course, schedules of release on a daily basis will probably work out best from the standpoint of rearing practices also, as it is likely that the production of male pupae will be almost a regular and even function.

The advantage of release of pupae on the ground is that containers can be placed where the emerging flies are likely to stand most chance of living, and of finding wild females. In a ground release, containers of pupae can be placed under shade or cover, so that the pupae do not suffer adversely from exposure to sun or other conditions that might hamper eclosion. Containers developed must be such that predators cannot destroy the pupae before they eclose.

It may be necessary at some release points to continue the use of marked flies as a portion of those that are released. The marking of pupa can be accomplished by dusting the pupa with a material that sticks to the ptilinum of the emerging adult and then is drawn back into the head as the adult fly breaks out of the pupal case. This would mean that sampling of flies in those areas must be done without replacement, since the adult flies collected will have to be taken back to the laboratory to be examined before it can be determined whether or not they were marked. If numbers of flies in the environment are relatively low, sampling without replacement could have significant effect upon the population levels.

3. Logistics

A field test of the scope of that envisaged requires careful coordination and planning of logistical elements. The logistics of a large scale field test become as important as the conduct of the test itself inasmuch as failure to provide proper elements at any point in time can negate the scientific findings that the test might have produced. It is suggested that a carefully drawn PERT chart of the logistic elements in the field test, as well as appropriate PERT-charting of all aspects of the study leading up to the field test be done to help assure the timely completion of tests upon which subsequent actions depend. Ideally, it would appear desirable to have assigned a person who is responsible for all the logistic requirements of the field operation.

a. Development of Data Forms. Data forms for all of the different kinds of measures should be developed early in the program. The data form itself is a stimulus to procedures that must be taken preliminary to recording the data. That is, a well constructed data form beforehand enables one to better plan the procedures which are to be recorded. For example, there must be separate data forms on such things as climatic conditions day-by-day in the test area; fly population density by sex, species, and marked or unmarked flies; release records, to include numbers marked and unmarked; insecticide treatments by time and amount; counts of the availability of host animals, both domestic and wild; incidence of trypanosomiasis in host animals, etc. For all forms, responsibility must be fixed so that it is known who collected which data when. It is suggested that the creation of data forms be given early priority in the study, well before the forms are actually needed.

b. Training of Data Gatherers and Pre-Test of Data Forms. Forms need to be pre-tested at least once before the experiment begins. Also, those persons who are going to be using the forms need to be trained in the kind of information to enter, if the form itself does not make that clear. Very often, ambiguities and misunderstandings of data requirements can be resolved in a conference session of data gatherers (with the forms), before ever going into the field.

c. Schedule of Materiel Requirements. In combination with the PERT-charting of activities, there needs to be a scheduling of materiels associated with each activity, so that such things as aircraft requirements, vehicle requirements, the need for release containers, transport containers, and so on, can be properly anticipated. Also, many operational aspects of the conduct of the field study that will need to be taken into account, such as housing for field personnel, feeding requirements, emergency medical care, etc.

d. Personnel Requirements. This aspect of the field test has been taken care of somewhat in the planning documents already prepared, and as part of the project agreement. The various skills required of personnel may not be so clearly defined. Over the 2-year period between now and the intensive preparation for the field test, the skills of personnel will become more evident, and appropriate assignments of personnel can be made at the time when these skills are needed.

e. Cost Records. Records need to be kept in such fashion that it is possible to cost out various aspects of rearing of flies, release procedures, and insecticide treatment, all separate from those aspects of the study that are completely experiment-related. That is, cost records should be broken out in a fashion that it is possible to extrapolate with confidence the cost of applying male release methods to other geographic areas and to those different in scale from the field experiment now planned. Cost should not be so global that it is impossible to differentiate between that portion that is experiment-related and the portion that is insect-control-related. Obviously, it is the hope of managers of this extensive project that the techniques being demonstrated and tested can be applied widely and effectively in other parts of Africa.

4. Evaluations

The evaluation of the results of the study should show two main points: (a) The effect of the experimental treatments (release of sterile males, and insecticidal control) upon the density of tsetse flies in the

test area, and (b) the effects of the experimental treatments on the incidence of trypanosome infestations of livestock grazing over the test area.* There may be a third result which needs to be demonstrated--that is, the cost effectiveness of these methods of control and their applications in other areas.

The principal evaluation must describe changes in fly concentrations over time as related to flies released over time, and to insecticidal treatments. Additionally, the effect of unintended and unexpected events, such as unusual weather conditions or changes in availability of hosts, etc., must be taken into account.

It is not clear in the project plans for the study whether sufficient effort is being allocated for evaluation and documentation of the results of the study. The documentation will require considerable time and effort, and it may be necessary to build into the project plan (reference Table 1) a separate major activity, "Evaluation and Documentation of Results," on a level with Rearing, Techniques and Methods, Field Population Studies, and Release Programs. This activity should be being thought about now, and an outline in the form of the probable concluding report be developed now, so that responsibilities be assigned among the current project staff to see that the writing and documentation begin very early in the process.

It is presumed that some of the project staff will remain after the fly release portion of the experiment is complete. There will need to be follow-up counts of fly populations for at least 6 to 12 months. Ideally, the test area should be under surveillance for 2 or more years to learn what the long-term effects of the experiment are.

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*This can best be determined by sampling young calves for the presence of trypanosomes. If the control is effective, then parasite rates should be minimal or significantly reduced.

IV. SUGGESTED PRIORITIES

It appears to the authors that there are six issues of major concern that need to be addressed by project personnel. They are arranged roughly in order of priority.

1. Learn the population dynamics of the probable test area, with respect to the numbers, species, locations, and seasonal fluctuations of flies, to include mark-release-recapture methods so that absolute numbers of flies per unit area can be described.

2. Develop sampling methodology with different counting techniques so that good estimates can be made of the proportion of flies per unit area that can be collected by various sampling methods; this knowledge is critical to planning for the numbers of flies that need to be reared for release.

3. Develop rearing techniques to levels that will provide sufficient numbers of excess flies for release, optimizing rearing methods and most viable and productive strains of tsetse, and planning for backup resources as may be necessary.

4. Establish agreement as to possible limited objectives for the project to face with the host country the probability that the availability of resources, such as flies, money, equipment, personnel may impose restrictions that make contingent objectives, and expectations, for the project mandatory.

5. Focus to an experimental design and alternative design that take into account the items noted previously, and then systematically plan for the conduct of the study under as rigorous conditions as the situation and resources permit.

6. Prepare a documentation plan, so that topics and responsibilities can be assigned as early as feasible to assure timely and complete reporting of the project.

APPENDIX A

STRAWMAN EXPERIMENTAL DESIGN

by

Floyd I. Hill

APPENDIX A.

STRAWMAN EXPERIMENTAL DESIGN

A. GENERAL

The purpose of a "strawman" design is to provide an explicit structured basis for consideration of potential alternatives. It shows one concept that follows the considerations provided in the "Preliminary Field Trial Design." Such a strawman does not represent a recommended design because its assumptions are not necessarily those that will obtain at the time of testing. The concept selected is one that will achieve the objective of providing an adequate test of the effectiveness of the Glossina morsitans sterilized male in controlling the G. morsitans population. The strawman concept outlined is flexible in its requirements for sterilized tsetse flies, field personnel, airborne equipment, navigation equipment, area isolation equipment, and effort.

The strawman design does not provide for a morsitans-free Mzeri Hill Ranch at the end of the test, or an isolation of the ranch by a barrier made by sheer clearing or a persistent insecticide. The requirements for any test of the sterilized male tsetse release concept preclude elimination of the fly over the entire ranch until after the test is completed; the presence or absence of a barrier during the test appears largely irrelevant to the adequacy of a sound experiment. If a tsetse fly-free isolated ranch is a necessary concomitant at the end of the test, the best way to achieve it is by treatment of the whole ranch after the test has been completed. If a barrier is desired, it can be constructed either after the test or during the test on a noninterference basis.

This "strawman" experimental design, therefore, treats the experimental objective of measuring the effectiveness of the sterilized G. morsitans independently of the other desirable objectives. The following plan is given in terms of an explicit set of the test objectives, measures of effectiveness, criteria, test environment, test procedures, instrumentation and fly release plan, and data analysis plan.

B. OBJECTIVE

To measure under operational conditions the effectiveness and cost of the use of sterilized G. morsitans tsetse fly males in control of the G. morsitans population in environments similar to that presented by the Mzeri Hill Ranch in Tanzania.

C. MEASURES OF EFFECTIVENESS

1. The differences as a function of time between the numbers of female G. morsitans measured in areas which have had sterilized male release to achieve a given sterilized/nonsterilized male ratio and those in which there was no sterilized male release as measured by:

a. fly rounds

b. standing catches

c. trap catches

2. The monetary cost (exclusive of experimental considerations) to achieve a given decrement of the number of female G. morsitans per unit area, and to maintain this decrement per unit area per unit time also as measured by the three methods just noted.

3. Discussion: The measures imply a series of conditions that should be observed in the test procedure:

a. Reduction of the G. morsitans population in the treated and control areas* to a number where it is feasible from the resources of sterilized males available for release to establish a satisfactory ratio of sterilized to unsterilized males (say 5:1) in the treated areas.

b. Measurement, before sterilized male release and after application of a nonpersistent insecticide, of the G. morsitans population in both the treated and the control areas by the three measurement techniques.

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*A treated area is one in which sterilized males have been released. A control area is one where there has been no release, but is the same in all other respects. Thus, if nonpersistent insecticide is used on the treated area, it is also applied to a control area at the same time. If measurements are made in the treated area, they are made in the same manner and with the same frequency as in the treated area. If ranch operations occur in one area, such operations should occur in the other, etc.

c. Contiguity of treated and control areas.

d. Rerelease of all flies captured during each measurement.

e. A measurement by all methods on the treated and control areas at approximately six-week intervals after the first fly release and at six-week intervals thereafter. Each of these procedures is developed in more detail in the test procedures section.

4. The three measurement methods are used according to the following rationale:

a. The primary measurement technique will be the fly rounds. However, there is believed to be a bias toward attracting a disproportionate number of males in fly rounds. Thus, the analysis of effectiveness is based on the reduction in numbers of captured females but the sterilized male G. morsitans release is based on the number of captured males.

b. Standing catches and fly traps are used as a check of the fly round measurements and as a tool to estimate the uncertainty of the measurement method.

c. Fly traps are used as a backup measurement for both other measurement techniques.

D. CRITERIA

1. A change in the total numbers of female G. morsitans due to sterilized fly release is indicated over an easurement interval, if the difference between the change (as made by the hree methods) in the treated and control area has an x percent probability of not being due to chance.

2. There is no criterion for the cost measure.

3. Discussion: Use of three methods of measurement allows estimation of a mean and a measurement technique error term to be associated with each measurement of female G. morsitans in the treated and untreated areas.

Since each measurement technique consists of several observations for each treated and each control area, variations in these observations also provide an error term associated with each technique.

E. TEST ENVIRONMENT

Figure A1 is schematic showing a three-phased treatment of Mzeri Hill Ranch using alternate "paddocks" for control and release areas. The test arrangement is such that Phase I would be a complete test if the availability of sterilized flies and test personnel were such that only one phase could be accomplished. If there were sufficient sterilized flies and test operating personnel, Phases II and III could be undertaken in sequenced time steps. For instance, each operation on subsequent phases would lag the previous one by one to four weeks, depending on the availability of sterilized flies. Thus, a test measurement crew (or crews) could operate in the Phase I area, and then in the Phase II area and Phase III areas prior to a second measurement in the Phase I area.

To give time for all operations prior to release of the sterilized male flies, it is desirable to space the beginning of the phases at least three weeks apart in any case. For this reason the spray boundaries are passed through portions of the ranch used for neither control nor fly release areas. The average paddock contains approximately 4.5 square miles. Four paddocks are treated in each phase, requiring sufficient sterilized flies to treat 18 square miles in each phase, with a 5:1 ratio of sterilized to unsterilized males. Assuming, say, that the Tanga Tsetse Research Project has a production rate of 10,000 sterilized male flies per month, then the test could operate effectively if the initial release per phase were 10,000 flies. Given this number, the first spraying steps would have to reduce the indigenous population of male G. morsitans to approximately 110 per square mile, or (assuming an approximately equal distribution of males and females) 220 as the total population of G. morsitans per square mile.

F. TEST PROCEDURE

Since the pupal development period (from fertilization to eclosion) is 5 to 6 weeks, the Phase I area should be sprayed three times at three-week

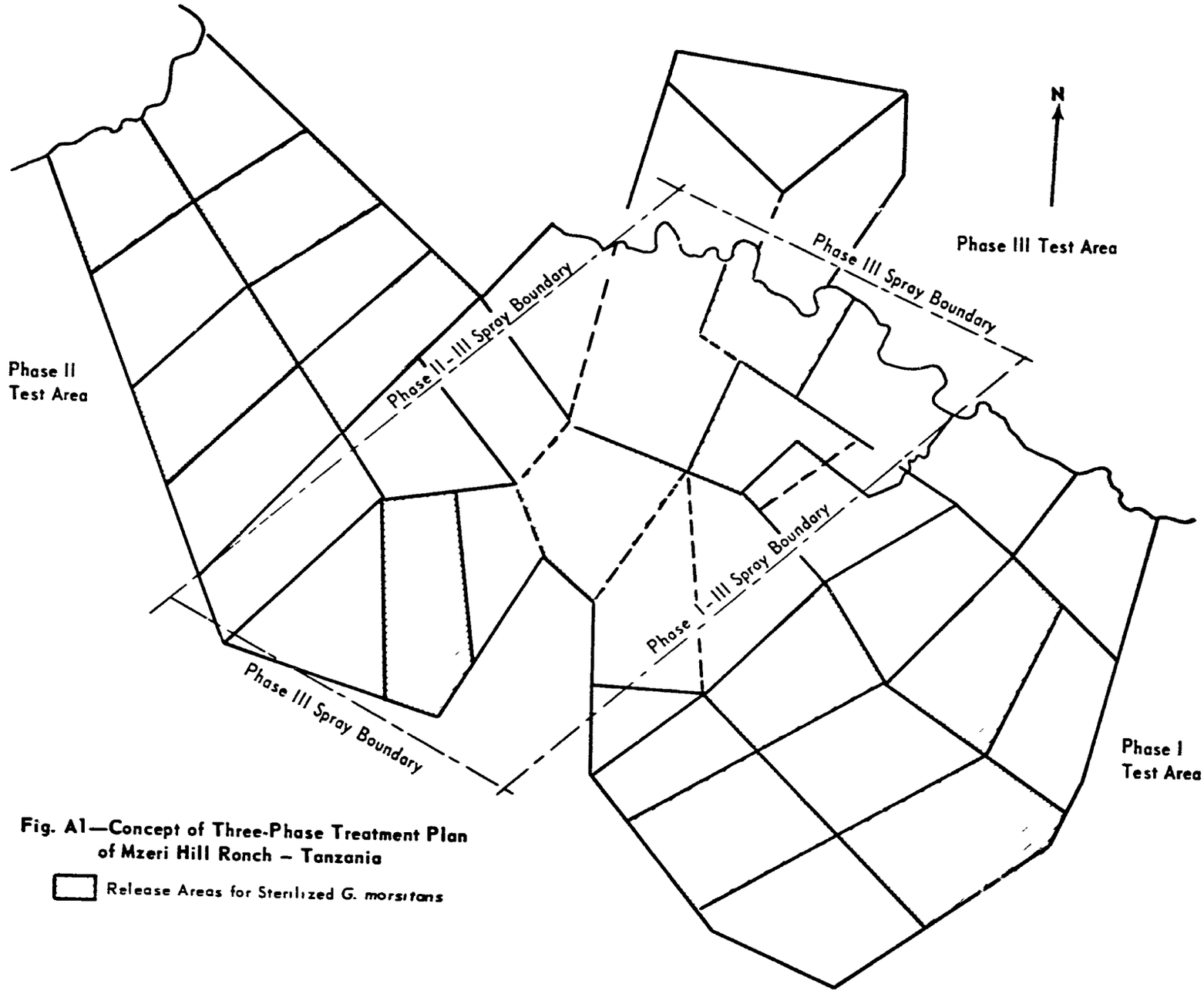

Fig. A1—Concept of Three-Phase Treatment Plan of Mzeri Hill Ranch – Tanzania

Release Areas for Sterilized *G. morsitans*

intervals with a nonpersistent insecticide. Two days after each spraying, measurement should be made of the fly count in each of the paddocks to be treated and to be controlled. Adjustment of the dosage between each spraying should be made to achieve a total fly count at the end of the six-week period to approximately 175-250 G. morsitans per square mile. Since the area to be measured in Phase I is of the order of 50 square miles, the measurement should be accomplished in a sequence that is to be held throughout the trial. After the last spraying and measurement, the sterilized male fly release should occur with a 5:1 ratio of sterilized males to residual unsterilized males in the four paddocks to be treated. Sufficient sterilized males should be available to treat at least one of the paddocks completely. Treatment of the other three can be phased as flies become available, say, at one-week intervals. Measurements on all nine paddocks should then occur at six-week intervals between each measurement on each paddock, and additional flies released after each measurement to maintain a 5:1 ratio of sterilized to unsterilized males. The test should continue through six cycles of fly release. When (and if), during the course of the test, measurement shows that the ratio has increased, the ratio of sterilized males to unsterilized males should be increased to 10 to 1 on each treated paddock showing such a result, and if greater than 10 to 1 to 20:1, etc.

Commencement of the test sequence on the Phase II and III areas will depend upon the fly production rate, the availability of measurement teams, and the availability of personnel to make the fly counts. Based on a production rate of 10,000 sterilized males per month, and availability of two trained measurement teams, the phases could be begun four weeks apart.

G. TEST INSTRUMENTATION AND STERILIZED FLY RELEASE PLAN

Since there is substantial uncertainty regarding the adequacy of the several measurement techniques, the use of three is planned. Figure A2 is a schematic test layout for the Phase I Test Area showing the paths for the fly rounds, the locations to be used for the trap catches and standing catches, and the locations for sterilized fly pupal release. The principal measurement instrument will be the fly round, with the trap and standing catches as correlative measures. The hypothesis is, however, that each

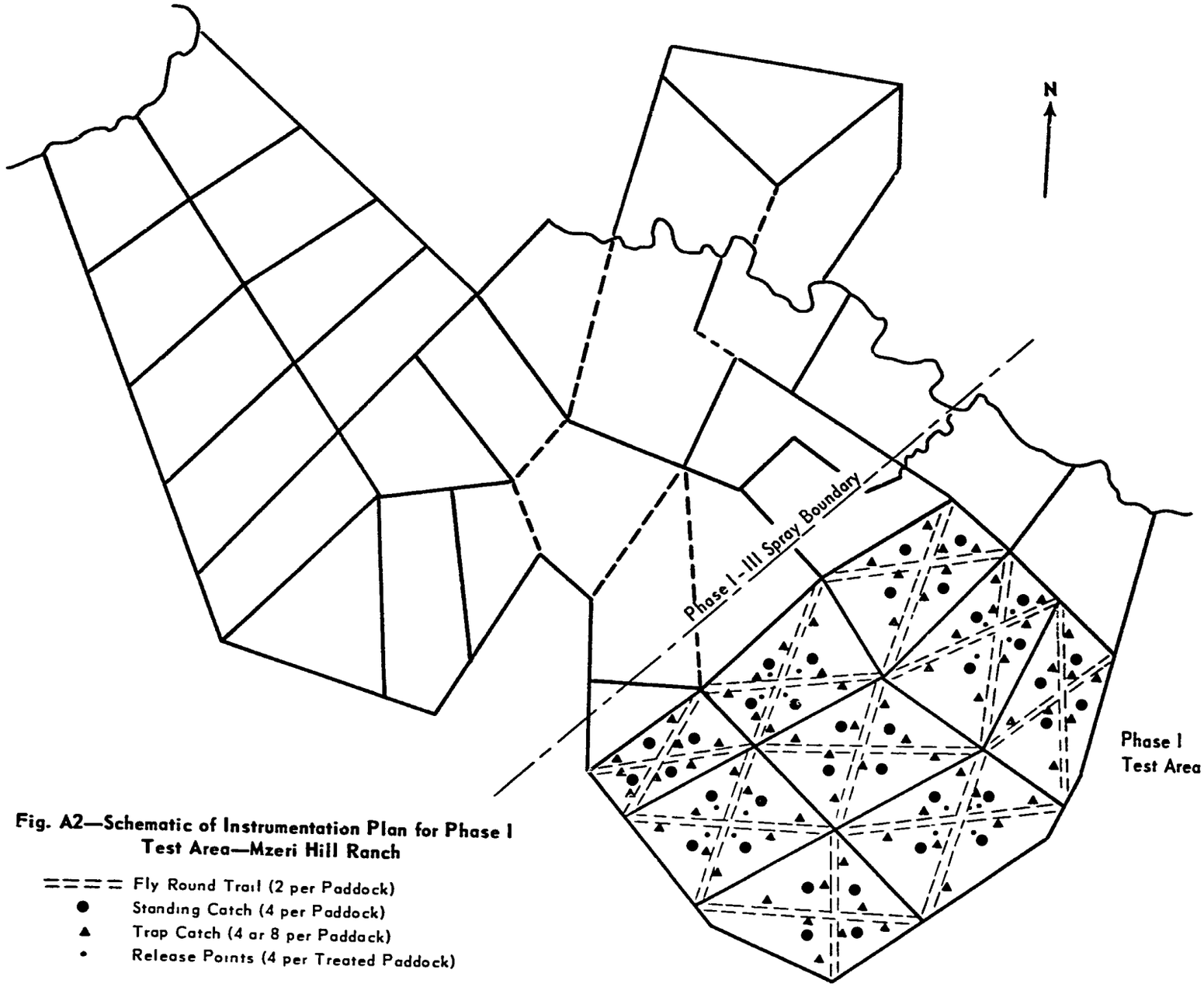

Fig. A2—Schematic of Instrumentation Plan for Phase I Test Area—Mzeri Hill Ranch

==== Fly Round Trail (2 per Paddock)
● Standing Catch (4 per Paddock)
▲ Trap Catch (4 or 8 per Paddock)
• Release Points (4 per Treated Paddock)

should measure the same differences in total fly population so long as the number of female flies caught in any one paddock remains about 10. This can be expected for at least the first three of the six six-week instrument cycles and should provide a relatively firm basis for estimation of the female G. morsitans population in the subsequent measurement cycles, where it is expected that the total number of female tsetse flies captured by the three measurement techniques will be substantially fewer than 10 in the treated areas.

The basic concepts underlying this layout shown in Figure A2 include:

1. Designated fly round trails. They, of course, are not likely to be straight as shown in the schematic, but should follow the terrain in a normal manner so that the measurement crews can walk easily. The doubled dashed lines are used to suggest there will be an attraction distance for flies as the walkers follow their marked path. Counts should be taken at at least six intervals along each trail. The counting point should be marked and remain the same throughout the test, and data on number of males and females attracted should be recorded by date, time of day, paddock, trail, counting point, and direction of travel. Direction of travel on trails should be reversed for each subsequent measurement on each trail.

2. Location of either two or four fly traps along each of the trails. Traps should be no closer to the trail than 150 meters. For test procedural convenience, it is desirable that the traps be near a fly round counting point. Thus, the fly round personnel after having dispersed the flies can service the trap, make a count, release the trapped flies, return to the fly round trail at their departure point, and resume the fly round.

3. Standing catches. These should be located approximately half-way between the fly round trails, approximately 500 meters from the trail intersection and 250 meters from the fly release points.

4. Fly release points should be approximately on the center of the axes between trails and 250 meters from the trail intersection. This

will place the release points at least 500 meters apart. Fly release must occur after all counts on each treated paddock have been completed and need not occur on the same day as the count. Perhaps most convenient would be a release two days after count completion to allow time for analysis of the data. However, whether the same day, the next day, or the day after that, the fly release time should always be at a fixed time after a count -- at the same time of day, and the same for all treated paddocks.

Figure A3 gives a strawman fly release plan based on a production rate of approximately 2,500 sterile male flies per week. Since approximately 2,500 male G. morsitans per treated paddock are assumed to be required for the first release, the sterilized fly releases are scheduled at one-week intervals. Although subsequent releases are unlikely to be limited by fly production, measurement (and hence sterilized fly release) are spaced so that release and measurement occur at six-week intervals. Also shown on the figure is the schedule of measurement of the control paddocks. The arrangement for the first week might be:

Monday	--	Third spray of Phase I test area;
Tuesday	--	Measurement of Control Paddock A;
Wednesday	--	Measurement of Treated Paddock 1;
Thursday	--	Measurement of Control Paddock B;
Friday	--	Fly Release, Treated Paddock 1.

In the following week it would then be:

Tuesday	--	Measurement of Control Paddock C;
Wednesday	--	Measurement of Treated Paddock 2;
Thursday	--	Measurement of Control Paddock D;
Friday	--	Fly Release, Treated Paddock 2, etc.

For a 2,500 fly production rate, Phase II could be interleaved with this schedule such that the first fly release in the Phase II area would be at 10 weeks after the initial spraying of the Phase I area. It is desirable that two measurement teams be trained in order that the three phases can be interleaved. With two teams, measurement in any one paddock

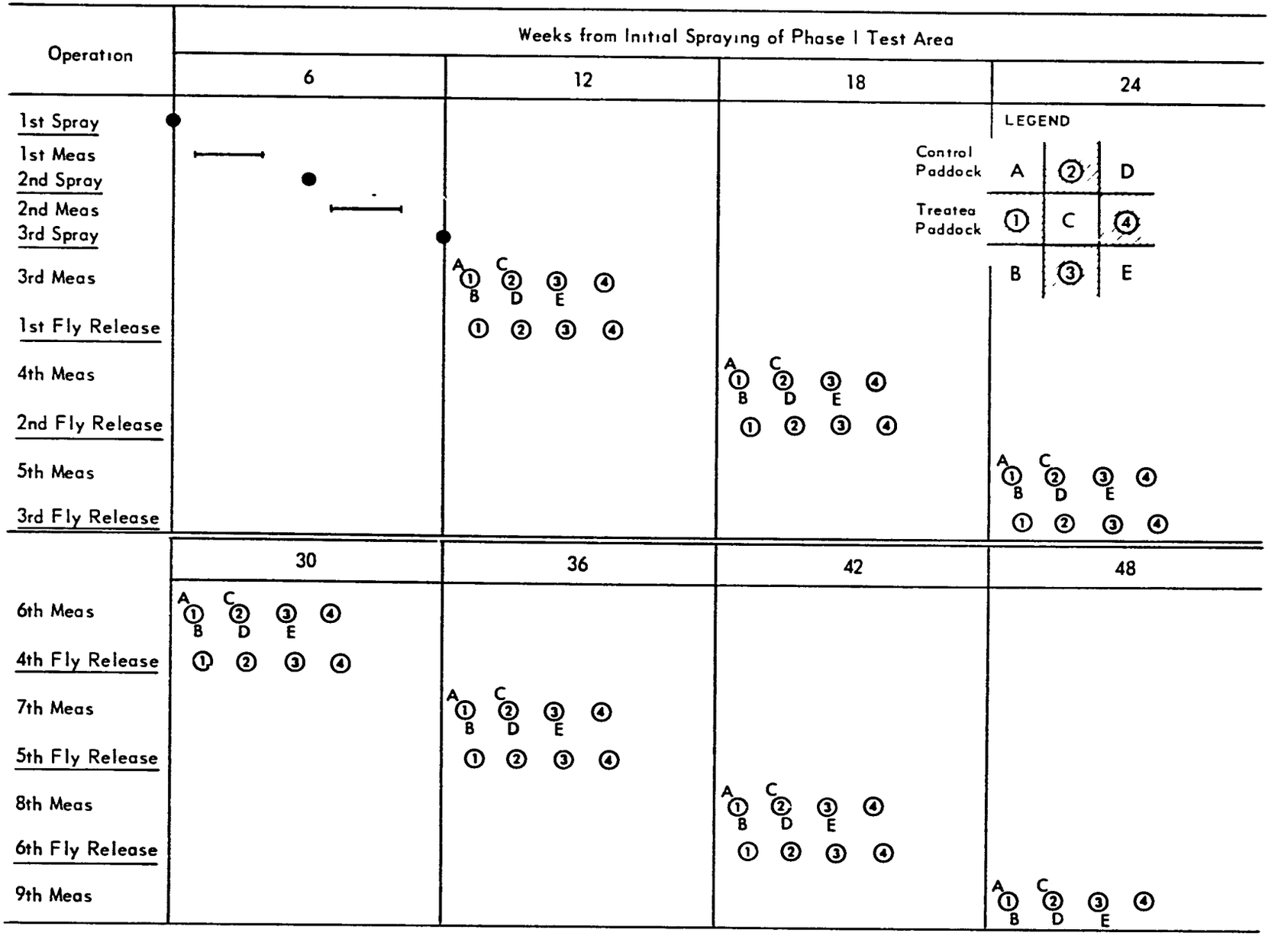

Fig. A3—Strawman Fly Release Plan—Phase I Test Area

Production rate of 2500 sterile male flies/week

should alternate between teams on successive measurements. It is also possible (by scheduling a six-day week in some weeks for these two teams) to interleave the Phase III Test Area measurements without an additional team.

H. DATA ANALYSIS PLAN

The data to be collected will be the number of male and female flies, for each of the three measurement techniques, in treated and control paddocks, as a function of time (six-week intervals). The fly count should be by type, e.g., numbers of G. morsitans, and of other tsetse fly species. The data record will also show the date, time of collection, location within the paddock, method of measurement and collection team. The basic measurement will be the number of unsterilized female G. morsitans captured.

The outline of the data analysis plan follows:

Let N_{fijp} = no. of female G. morsitans captured by measurement technique i on measurement j, on paddock p, and

N_{mijp} = no. of male G. morsitans captured by measurement technique i on measurement j on paddock p.

An estimate of k_i, the female to male ratio of captured G. morsitans by measurement technique i, can be made from the first three measurements made on the nine paddocks.

$$k_{ijp} = N_{fijp}/N_{mijp}$$

$$k_i = \sum_{p=1}^{9} \sum_{j=1}^{3} (N_{fijp}/N_{mijp})/27$$

Tests should be applied to this hypothesis to determine if k_i differs significantly for paddock or measurement number (the fly count should change appreciably between the first and third measurements). If there is a significant difference between paddocks, but none in measurement number, then k_{ip} should be used. If there is a significant difference in measurement number but not in paddock, then k_{i3} should be used. If there is a significant difference in both paddock and measurement number, then the assumptions of measurement are invalid.

For purposes of continued discussion, it will be assumed that $k_i \pm \tilde{k}$ can be determined -- that is, paddock and measurement order are independent. Since there are three measurement techniques used, there is a good chance that k_1, k_2, or k_3 will have this property and that, for the standing catch, k will be ≈ 1. The ratio of male to female G. morsitans captured is used to measure the movement of sterilized male flies from treated to control paddocks. The hypothesis is that k_i for the control paddocks should remain constant (for all three measurement techniques) until invasion of the control paddocks by sterilized G. morsitans males. At which time k_i should decrease, since the preponderance of the invasion should be male.

A second control on this measurement is the ratio of female to male of other species of tsetse fly captured, which is expected to remain constant in both the treated and control areas. If the female to male ratio of other species of tsetse fly varies significantly in the treated areas, then there is an interaction of the G. morsitans with the other species, and the amount of this interaction can be estimated from the treated areas. Even if such an interaction occurred, it would be of only minor importance in testing the effectiveness of reduction of unsterilized female G. morsitans by sterilized male G. morsitans release.

The first fly release will be based on the best estimate of the unsterilized male G. morsitans population density of each paddock to be treated, ρ_{mjp}. If the three measurement techniques are held in equal regard by those expert in such measurements, then the best estimate of ρ_{m3p} is:

$$\rho_{m3p} = (X_1 N_{m,1,3,p} + X_2 N_{m,2,3,p} + X_3 N_{m,3,3,p})/3A_p$$

Where: X_1, X_2, and X_3 are the ratios of the number measured by the sampling technique to the total number in the area as measured by techniques 1,2, or 3. These numbers are presumed to be known from previous work using these techniques. The number of G. morsitans released on the first release to achieve a 5:1 ratio for paddock p is:

$$N_{r1p} = \frac{5}{(1-y)} A_p \rho_{m3p}$$

Where: y = the fraction of irradiated females in the fly release

A_p = area of the paddock

On measurement 4, the change in the indigenous female G. morsitans population for any treated paddock, p, by measurement technique i is:

$$\Delta_{fip}(3\text{-}4) = N_{fi3p} - N_{fi4p} \left[\frac{yN_{r1p}d_{f6}}{N_{fi3p} + yN_{r1p}d_{f6}} \right]$$

Where: d_{f6} = surviving fraction of sterilized female flies after six weeks.

For a control paddock, the change is:

$$\Delta_{fip}\ (3\text{-}4) = N_{fi3p} - N_{fi4p}$$

The effect of the treatment on paddock 1 between measurements 3 and 4 is the difference between Δ_{fi1} and $(\Delta_{fiA} + \Delta_{fiB} + \Delta_{fiC})/3$. (See Figure 3.) Similarly, the effect of treatment on paddock 2 is the difference between Δ_{fi2} and $(\Delta_{fiA} + \Delta_{fiB} + \Delta_{fiC})/3$. The overall effect of treatment is:

$$\Delta_{fi}\ (3\text{-}4) = \frac{5(\Delta_{fi1} + \Delta_{fi2} + \Delta_{fi3} + \Delta_{fi4})}{4(\Delta_{fiA} + \Delta_{fiB} + \Delta_{fiC} + \Delta_{fiD} + \Delta_{fiE})}$$

Significance tests of the difference in the mean case can be run by calculating the mean and variance of $\Delta_f(3\text{-}4)$, recognizing that there are 12 values of Δ_{fip} of the treated paddocks and 15 values of Δ_{fip} for the control paddocks, when all three measurement techniques are considered.

The estimate of the number of flies to be released in the second release of sterilized G. morsitans (right after measurement 4) is as follows:

$$\rho_{m4p} = \frac{(X_1N_{m1,4p} + X_2N_{m2,4p} + X_3N_{m3,4p})\ N_{r1p}\ (1-y)\ d_{m6}}{(X_1N_{m1,3p} + X_2N_{m2,3p} + X_3N_{m3,3p} + N_{r1p}\ (1-y)\ d_{m6})\ 3A_p}$$

Where: ρ_{m4p} = estimated density of unsterilized male G. morsitans at measurement 4 on paddock p.

d_{m6} = surviving fraction of sterilized male G. morsitans after six weeks.

The number of sterilized G. morsitans released after measurement 4 (Release 2) is for any treated paddock p:

$$N_{r2p} = \left[5A_1\rho_{m4p}/\ (1-y)\right] - N_{r1p}d_{m6}$$

Subsequent releases will follow the same relations using a different mortality rate applied to those sterilized G. morsitans released at different time periods. Thus, for the third release after measurement 5:

$$N_{r3p} = \left[5A_1\rho_{m5p}/\ (1-y)\right] - N_{r1p}d_{m12} - N_{r2p}d_{m6}$$

Where: d_{m12} = surviving fraction of sterilized male G. morsitans after 12 weeks.

I. DISCUSSION OF STRAWMAN DESIGN

The purpose of a strawman experimental design is to explore one option among several possible designs to determine what data are needed and what operations are needed to get these data. It is a useful framework for considering other designs in the light of difficulties that might be encountered with the implementation of the strawman design. The key consideration is: Does a design different from the strawman remove such difficulties without introducing new ones of even greater difficulty? An example is the effect of movement of domestic and wild animals across paddock boundaries. In the strawman design the effect is balanced out so long as the movement is random. Systematic movement of domestic herds would require a different analysis plan and a careful record of those movements. It should be noted that use of the female to male capture ratio, k_i, for both the G. morsitans and other species of tsetse fly should provide for all systematic movement (on a time basis) of the G. morsitans.

Another alternative would be to treat large areas (say, half the ranch) and leave half the ranch for control area. If this occurred, then the problems of weather variations and animal movement would be exacerbated rather than ameliorated. The requirements for sterilized

G. morsitans would either be increased or the residual numbers of flies left after spraying would have to be small indeed, and the number of female G. morsitans captured would be yet smaller. Variations in the fly count for different paddocks would still be important, but estimation of differences would be difficult for the very small numbers of captures involved. The concept of a barrier about the treated and control areas is useful from an operational point of view, but, without elaborating the test extremely to measure barrier effectiveness (actually setting up a different test of barriers), its presence or nonpresence would have to be ignored (by assumption) in the analysis of the effectiveness of the sterilized male fly in reducing the population of the G. morsitans.

The statement of a given test objective and effectiveness measure is a managerial decision as contrasted to an analytically developed concept. Those of this strawman may be inconsistent with the desires of USAID and the Tanzanian government. However, the strawman provides an explicit statement to which explicit exception can be drawn and its consequences can then be traced through the other necessary steps outlined in this strawman plan. For example, it may be desired to use reduction in trypanosomiasis in the Mzeri Hill Ranch herds as an effectiveness measure. This involves a completely different experiment and an indirect measurement of the reduction of the number of unsterilized female G. morsitans. Correlation would be much more long-term, since it is presumed that the sterilized flies would continue to transmit the trypanosome. While the measures of effectiveness proposed on this strawman are not in the province of analysis, they do have the analytically convenient virtue of measuring the most immediate effect sought by the release of sterilized male G. morsitans.