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REPORT ON TSETSE RESEARCH PROJECT

TANGA, TANZANIA.

UNITED REPUBLIC OF TANZANIA

AND

UNITED STATES AGENCY FOR INTERNATIONAL DEVELOPMENT.

PROJECT No.931-17-130-030-73. AGREEMENT No.72-931-1.

PERIOD: July 1 through December 31, 1974.

Submitted by:

D. Leroy Williamson

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE.

Office of Agriculture
Bureau for Technical Assistance
Agency for International Development
Washington, D. C. 20523

SUMMARY

A record number of field-collected pupae were obtained by increasing manpower in a preselected area during an optimum period when our records showed most success in finding the largest number of viable pupae. Laboratory colonies are now self-sustaining at a level that no further field collections will be required to help meet target dates for stock colony expansion (page 1).

On December 31, a total of 17,405 Glossina m. morsitans were maintained on goats and rabbits. During that month fertilized females of the 'Tanga' strain produced 19,829 pupae. Good performance of nucleus stock colonies was maintained (page 2). Best performance of Tanga strain colonies continued in the Internal colony. This colony has been self-supporting for approximately one year (pp. 3-4). The data indicate that continued improvement in rearing success will occur as flies become laboratory adapted.

An experimental colony was initiated in late November consisting of a genetically produced mutant strain of distinctly different color from wild G. m. morsitans (page 4). Their usefulness as a marked insect in field surveys will be investigated.

Host animals were increased through purchase and the project breeding program. Through selection, larger and more docile goats than can be found locally are now used. Different systems were tested for introducing new animals into the fly feeding system (page 5). Each goat will feed 500 flies per feeding day assigned. Host suitability tests were continued and animals on which less than 90% of the flies fed were eliminated (page 6).

Introduction of domestic rabbits in the tropics as an alternate host for fly feeding was so far successful. Breeding experiments for tropical hardiness and ear size were performed and offspring are being tested (page 7). After 8 weeks exposure to local environmental conditions, no mortality has occurred. Elimination of air conditioned rabbitries would be advantageous.

Surveys were concluded in determining the most suitable test area for sterile male releases. Mzeri Hill Ranch, with the exception of being isolated, has the characteristics sought for a field trial area. A practical means for constructing a tsetse-fly barrier around the test area will be determined (page 8).

The magnitude of construction and maintenance requirements for laboratory facilities and the shortage of required Tanzanian personnel is the primary limitation of more rapid fly colony expansion and research progress (pp. 9-10). Remedial action continued to receive high priority.

A Review Committee was organized and met at Tanga for the first time in September. The local research team and the project benefitted from this consultation and continued participation by this group is highly recommended (page 10).

This Semi-Annual Progress Report should be read in conjunction with the report covering the preceding six months period. Reorganization of the project and personnel was initiated at that time by a different team of expatriate investigators and Tanzanian officer counterparts. Results and status of expanded activities outlined in that report are presented here under similar headings along with recent developments and future plans.

REARING ASPECTS

Pupae Collection for Colonization

Records of the number of pupae collected and percentage eclosion in the laboratory are available since 1972. From these data and our present staff experience, collection efforts were concentrated during the period when most pupae could be located in the field and when eclosion percentage was highest. September and October were optimal months in conforming to these criteria. Ten additional men were employed as casual labourers and placed with the experienced crew of 12 men. This effort resulted in a record production of pupae/man/day and the addition of 19,866 useable flies to the External I laboratory colony. The handling of field collected pupae, i.e. refrigeration and transport procedures, were the same as previously reported. Laboratory colonies are now self-sustaining at a level that no further field collections will be required to help meet target dates for stock colony expansion.

In conjunction with pupae collection, comparative information on pupae abundance and degree of parasitism between Mzeri Hill Ranch, the proposed sterile male release test area, and Swagilo were obtained. The Swagilo collection area is located some 60 miles southwest of the ranch. In August a 14 man team collected pupae at Mzeri Hill to determine the suitability for collecting pupae from that area where other work was in progress. This would reduce travel and allow more efficient coordination of field activities. A portion of the ranch searched was not as productive as Swagilo. In September two crews, comparable in experience, were organized and one crew sent to Mzeri, the other to Swagilo to compare results during the same period of collection. The results are shown in Table I. Only 14 pupae/man/day were obtained from Mzeri Hill compared to 104 pupae/man/day from Swagilo. Eclosion from Mzeri Hill pupae was lowest due to the presence of the parasite Thyridanthrax sp. which was rare in pupae from Swagilo. Thus, in October our collections were from the Swagilo area which allowed maximum collection efficiency and the largest number of useable adults for colony expansion.

TABLE I

SURVIVAL AND ECLOSION OF FIELD COLLECTED PUPAE OF G.M.MORSITANS HELD AT 78°F ± 3° AND 70% R.H. ± 10% AT TANGA

Date	Collection Site	Number Collected	Pupae/Man/ /Day	Number Emerged	% Eclo- sion	% Para- sitism	% Mortality Other Causes
Aug 1974	Mzeri Hill	2,200	40	1,650	75%	7%	18%
Sept 1974	Mzeri Hill	1,330	14	745	56%	14%	30%
	Swagilo	11,400	104	8,094	71%	0	29%
Oct 1974	Swagilo	18,445	115	12,542	68%	0.02%	32%
	Total:	33,375		23,031			

Performance of Laboratory Colonies

In the span of six months the 'Tanga' strain laboratory fertilized female stock colony increased 3.9 fold, with the performance trend continuing upward. On December 31, a total of 17,405 male and female G. m. morsitans of all colonies were maintained on goats and rabbits. The number of fertilized females in each of colonies was: Internal 5,292, External II 7,034, External I 761, and Rabbit 555. In addition, experimental colonies designated Bristol and Mutant Strain each had 499 and 25 fertilized females, respectively.

TABLE II

MEAN DAILY PERFORMANCE OF G.M.MORSITANS COLONIES IN TANGA AT 24°C ± 1° AND 70% R.H. ± 5%

1974 Month	Colony	Mean Number of Fert. Females	% Daily Mortality of Fert. Females	Number of Pupae per 100 Females
May	Internal	1126	1.40	6.9
	External II	900	1.93	5.6
	External I	690	2.54	5.0
	Bristol	527	0.64	8.9
	Rabbit	-	-	-
June	Internal	1547	1.11	6.1
	External II	1253	1.33	5.7
	External I	168	5.17	4.1
	Bristol	577	0.85	7.5
	Rabbit	266 *	0.83	5.9
July	Internal	2031	1.13	5.4
	External II	1575	1.53	5.0
	External I	-	-	-
	Bristol	921	1.18	6.5
	Rabbit	321	0.89	5.4

* Recording started June 11, 1974.

Table II continued

1974 Month	Colony	Mean Number of Fert. Females	% Daily Mortality of Fert. Females	Number of Pupae per 100 Females
August	Internal	2514	1.31	5.2
	External II	1679	1.69	4.8
	External I	300	0.70	0.6
	Bristol	1132	0.89	7.4
	Rabbit	396	1.03	6.9
Sept	Internal	2851	1.48	5.9
	External II	1829	1.78	5.3
	External I	762	1.47	3.7
	Bristol	717	0.76	7.3
	Rabbit	520	0.87	7.7
October	Internal	3216	1.45	5.5
	External II	2337	1.34	5.0
	External I	3039	1.59	2.4
	Bristol	601	1.28	8.5
	Rabbit	595	0.83	8.1
Nov	Internal	3930	1.26	5.1
	External II	3578	1.33	4.6
	External I	4211	2.27	4.2
	Bristol	450	1.84	6.1
	Rabbit	561	0.88	7.5
Dec	Internal	4798	1.16	5.5
	External II	5687	1.13	4.5
	External I	1698	3.31	4.2
	Bristol	457	0.40	6.8
	Rabbit	568	0.48	8.4

Tests were continued to determine peak emergence of females as an indicator of suitable climatic control in the insectary. All colonies on goats were tested simultaneously on a given date. The range in peak emergence of females from pupae in the different colonies deposited May 30th was 30-31 days; July 3, 32-33 days; August 7 was 31-32 days; September 18 was 31-33 days; and November 12 was 31-32 days.

Performance of the External II colony in terms of pupae production has never been as good as the Internal colony (Figure I). Handling procedures are the same for both colonies housed in the same laboratory. The main difference in the two colonies is that F_1 pupae from the External I colony are added to the External II colony. The difference in performance of the two colonies can be primarily attributed to differences in degree of laboratory adaptation. This is further substantiated by the still lower performance of the External I colony derived from field collected pupae. Differences in longevity of fertilized females in each of the colonies is shown by Age Group Period in Table III.

FIG. 1: AVERAGE NUMBER OF PUPAE/FERTILIZED FEMALE DURING LIFE SPAN IN INTERNAL AND EXTERNAL II COLONIES.

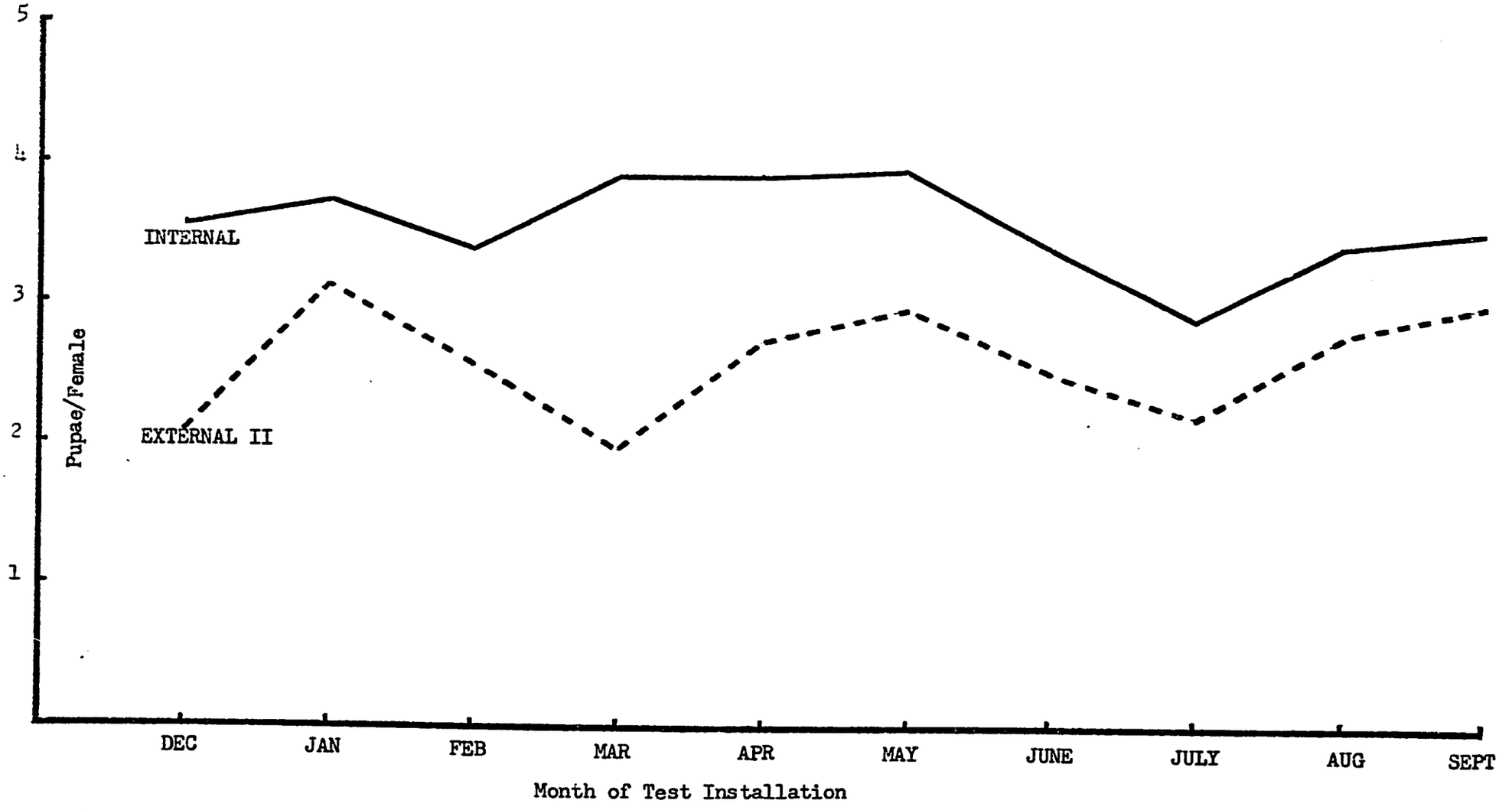


TABLE III

PERCENT OF FERTILIZED FEMALE G.M. MORSITANS ALIVE AT THE MIDDLE OF CONSECUTIVE 9-DAY AGE GROUP PERIODS

9 Day AGP	Internal	External II	External I	Rabbit	Bristol
1	97.8	96.6	90.8	90.5	98.2
2	92.9	90.9	82.6	83.1	94.1
3	89.6	85.4	75.3	80.3	90.3
4	83.4	77.7	63.0	78.4	86.7
5	76.9	67.6	48.5	75.8	81.5
6	67.4	55.8	32.8	73.1	76.2
7	52.7	41.3	21.1	69.3	69.4
8	39.6	28.1	13.3	63.3	58.6
9	26.6	16.9	4.7	55.8	45.6
10	14.1	10.0	-	47.9	35.0
11	8.0	6.9	-	45.6	23.1
12	4.5	5.2	-	39.0	16.2

A near tragedy to the rearing effort was detected and resolved, but resulted in a period of lower productivity in the colonies in June, July and August. Fleas and lice appeared to be increasing on goats housed in the insectary and the animals were washed with chloroform. However, the goats were not washed with soap and water after treatment. A toxicant in the chloroform was accumulating on fly cages during the daily feeding routine and was slowly poisoning the colonies. Flies placed in beakers after complete evaporation of chloroform contents died within a few hours. Remedial action included the removal of a flea-infested uniport near the insectary, removal of known contaminated cages, and a 24 hour soak and wash of all cages used in the insectary in a solution of 5 c.c. Teepol/litre of water. This could have been an unfortunate circumstance, however, it served to make known to all staff members the requirement of constant vigilance and strict adherence to prescribed rearing procedures.

Introduction of Mutant Strain

Upon request from researchers at University of Amsterdam, we supplied 200 pupae collected from the field in Tanzania and 200 pupae from our Bristol colony to assist them with their studies on isoenzyme polymorphism in tsetse flies. In return, we received 49 pupae on November 27 from a conspicuous mutant stock developed in their laboratory. The mutant strain was developed using pupae of G. m. morsitans from Dr. Itard's laboratory in France and are of Rhodesian origin. The adults are easily distinguished from the wild type by their paler coloration. This is interesting to us from the standpoint of having "marked" insects for field studies.

A colony at Tanga was started using rabbits as hosts and will be expanded. Tests will include mating compatibility with our Tanga strain, as well as performance comparisons in the laboratory and field. A summary of the initiation of the mutant colony and status as of December 31, 1974, is as follows:

Emergence from 49 original pupae:	22	♂♂ and 25 ♀♀
Number of females mated:	25	
Number of fertilised females alive:	25	
Number of pupae produced:	40	

HOST ANIMALS

Goat Husbandry

The laboratory herd was increased from 150 to 225 animals through purchase of improved breeds and our own breeding program. Animal health has remained good since changes described in the previous semi-annual report were instituted. Sixty goats were purchased from Bamburi Portland Cement Co., and Edward Rodwell & Co., in Mombasa, Kenya, and 15 animals obtained from the local breeding system.

The need for host animals increased sharply as a result of improved fly colony performance. A test was conducted using 3 to 4 month old kids to determine their response to shortened introduction for fly hosts (Table IV). The technique consisted of varying the number of flies allowed to feed on the goats. The feeding system is tabulated below. There was no difference in weight gain between the test goats and control animals.

TABLE IV

DIFFERENT FLY FEEDING LEVELS APPLIED TO 3-4 MONTH OLD GOATS

Goat	Number of Flies Applied on Consecutive 3-day Intervals														
A	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75
B	5	5	10	20	30	40	50	60	70	75	75	75	75	75	75
C	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75

The skin of Goat C thickened and indicated that an acceptable limit of fly feeding on the animal had been exceeded. The disposition of Goat A was poor compared to Goat B in that the animal was restive during feeding. However, Goat A and B showed no external signs of skin reaction.

The test was not repeated and, in fact, we abandoned the idea of gradual introduction of young animals into the fly feeding herds to conserve time, space and personnel. Young animals will be weaned and removed from the insectaries until they reach one year of age. After this time they will receive an abbreviated introduction to the fly-feeding regime. A system was tested using mature goats purchased from Mombasa, whereby the number of flies feeding on each goat every third day was increased by the following increment: 25, 25, 50, 75, 100, 200, 300, 350, 500. A total of 61 animals were treated in this manner with only 5 animals showing skin reaction (thickening). Thus, a plan was designed to place mature animals capable of feeding 500 flies inside our insectaries for maximum use of the facilities. When weaned animals reach maturity (age one year) they will be returned to the insectaries and inducted into host herds as described above.

Host suitability tests were conducted through September, with approximately 75 percent of the animals tested. The tests were interrupted due to a shortage of cage netting material. By December 29, these tests were resumed using 2-day old males from the Internal, External II and Bristol colonies. The technique remained the same as previously described. Ten animals with poor suitability results (less than 90% fed flies) were culled and 10 goats with better than 93% rating were culled becaused of bad temperament and small size.

Consultants have recommended that goats receiving medication be rested for 3 weeks. A test was conducted consisting of 3 replicates of treatment with Thiabenzole (an antihelmintic compound) and controls to determine if treatment with this compound could be used without interruption to the feeding regimen (Table V). Two dose levels are prescribed by the manufacturer; a 'normal' dose and a larger dose for severe parasitism. Test animals received the dose of Thiabenzole recommended for severe parasitism (30 mg/pound body weight) three days prior to use for fly feeding. Control animals received an equal volume of water. The results revealed no significant difference in longevity and fecundity of G. m. morsitans fertilized females, fed on the animals. A 'normal' dose of Thiabenzole could be safely used without adverse effects to the tsetse colonies and without removing animals from routine use.

TABLE V

PERFORMANCE OF G.M. MORSITANS FED FOR 60 DAYS ON GOATS TREATED WITH THIABENZOLE (30 MG/POUND) 3 DAYS PRIOR TO USE AS HOSTS *

AGP	Fly Performance	Treated	Control
1	No. Pupae	-	-
	No. ♀	303	303
	P/♀	-	-
2	No. Pupae	191	217
	No. ♀	296	293
	P/♀	0.645	0.741
3	No. Pupae	190	241
	No. ♀	279	284
	P/♀	0.681	0.848
4	No. Pupae	212	216
	No. ♀	266	269
	P/♀	0.796	0.803
5	No. Pupae	193	202
	No. ♀	249	248
	P/♀	0.775	0.815
6	No. Pupae	189	169
	No. ♀	214	227
	P/♀	0.883	0.744
7	No. Pupae	142	133
	No. ♀	201	192
	P/♀	0.706	0.693
Total	No. Pupae	1117	1178
	% ♀ Alive at 60 days **	0.663	0.634
	P/♀ **	3.686	3.887

* replicated 3 times ** "t" test not significant at o.05

Rabbit Husbandry

In addition to the original nucleus of experimental rabbits obtained from Vienna, Austria and Nairobi, Kenya (see previous report), 8 lop-eared (LE) type rabbits were received from Langford, England, in August, 1974. Of these original 26 rabbits, 21 remained alive in an air conditioned rabbitry at Tanga. The use of rabbits as an alternate host for G. m. morsitans rearing is contingent upon success in maintaining domestic breeds, particularly the lop-eared variety, in the tropics. The New Zealand White (NZW) rabbit, successfully reared in Kenya, appears to possess a degree of tropical hardiness, but we are more interested in the lop-eared variety from the standpoint of fly rearing.

Crosses of NZW and LE rabbits were obtained from which F_1 offspring had desirable ear-size and hopefully tropical hardiness. The largest average ear surface was obtained from LE♀ x NZW ♂ which almost equalled the LE x LE offspring (Fig. 2). These offspring and offspring of LE and NZW parentage have been removed from the air conditioned rabbitry to hutches in the goat barn of our insectary. After 8 weeks under these conditions, no mortality has occurred. This investigation will be expanded as more hutches, constructed locally, are completed.

SELECTION OF RELEASE SITE

The important matter of settling on a suitable release site was pursued during the past six months. As indicated in the last report, the previously arranged agreement to use Tanzanian Government-owned bulldozers for Mzeri Hill Ranch barrier clearing was rescinded. Means to obtain another approach to the construction of the barrier through consultation with USAID, TanGov, and bids from private contractors were pursued. Results of investigations to select alternatives were forwarded to the respective agencies.

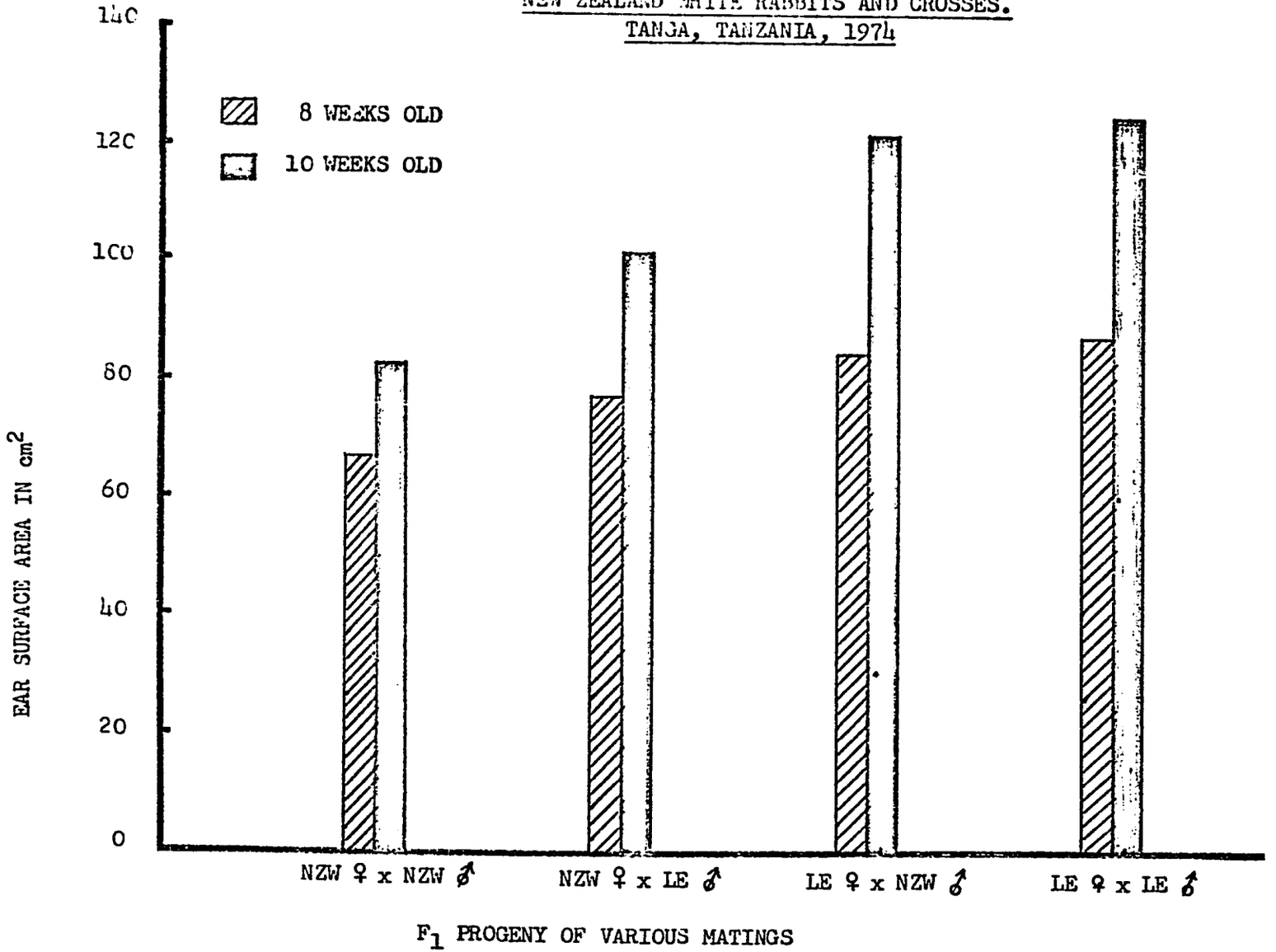
Mzeri Hill Ranch, with the exception of being isolated, possesses the characteristics sought for a sterile male release field trial. The size of the test area is adequate and comparatively near to Tanga; the Ranch Manager is amenable to our plans and cooperative, and other factors such as road networks over the ranch, disease incidence, G. m. morsitans populations, etc. are prime to adequately test the concept. For these reasons, an alternative barrier to sheer-clearing with heavy machinery was proposed. This would involve the use of defoliants (herbicides) and persistent pesticide in the barrier zone. This alternative has been verbally agreed to by officials of the Ministry of Agriculture.

As a recourse, surveys were made of Kilwa Peninsula. The peninsula of 50 sq. miles was isolated from the mainland by a mile wide barrier and treated with insecticide in 1970. G. m. morsitans and trypanosomiasis in cattle were identified from our survey of the area in early December, 1974. In the event no workable solution is agreed upon for Mzeri Hill Ranch, a release of sterile males at Kilwa Peninsula can be accomplished. Logistics will be more complicated due to its 430 mile distance from Tanga and its inaccessibility by road during the rain seasons.

LABORATORY FACILITIES

Our research progress was and will continue to be shadowed by the concomitant construction of laboratory facilities and shortage of Tanzanian personnel.

FIG. 2: COMPARISON OF EAR SURFACE AREA OF LOP-EARED AND NEW ZEALAND WHITE RABBITS AND CROSSES. TANGA, TANZANIA, 1974



Main Office Building

Additional needs for office space were not accomplished because priority was placed on completing and maintaining the insectaries. Additional offices will be constructed as soon as possible along with completing ceilings in a portion of the building. Six Tanzanian officers presently occupy one small office. Exterior painting of the building was accomplished through contract with a local painter.

Insectary No.1

This building is fully operational and was joined to a new sewage system. Work was initiated to install a Defensor humidifier in the fly holding room and a different water reservoir system.

Insectary No.2

This unit is ready for use with the exception of air conditioners. Units ordered 7 months ago were not received. Cement walkway to the building was constructed.

Standby Generator

The unit is fully operational and was repeatedly used during periods of power failure.

Irradiator Unit

Installation was completed inside the proper building. Dosimetry tests were performed by Dr. D.A. Lindquist, I.A.E.A.

Field Station

Three uniports on cement foundations were constructed at Mzeri Hill Ranch and work started on two prefabricated houses to be used as living quarters and laboratory.

Sewage System

A sewage system for both insectaries, including the animal barns, was constructed. The drain field was extended to elephant grass plantings to water and fertilize a portion of the pasturage.

Water Reservoir for Humidifiers

Work was started on a new system to pipe collected rain water to humidifiers. The present system requires daily filling of cannisters inside the insectary with water collected in cement tanks in front of the office. The new system will collect rain water from the insectary roofs into 815 gallon tanks connected directly to the humidifiers.

Animal Food and Pasturage

Rabbit food and goat food concentrates remained available in the country. Additional acreage for expanding animal forage was granted from Tanga Dairy. Expansion of pasturage is contingent upon locating water for irrigation.

Water Well Exploration

A test hole was drilled to a depth of 107 feet without success in finding water. Application to the Principal Geologist, Dodoma, was made in December for a geological survey to locate water for irrigation on project property.

ADMINISTRATIVE MATTERS

Personnel

The Tsetse Research Project staff as of December 31, 1974, consisted of 5 expatriates, 1 Field Officer, 6 Assistant Field Officers, 58 Field Assistants/Artisans and 6 casual laborers. One additional Assistant Field Officer is being processed to join our staff, but if received our A.F.O.s will remain the same due to notification that one of our present A.F.O.s must report for National Service in mid-January. One additional expatriate Entomologist is expected to arrive in late February. It has been repeatedly stressed that we cannot operate our research with casual labor. Efforts to correct a growing deficiency of counterpart trainees and functional staff must continue to receive high priority.

Review Committee

A Tsetse Research Project Review Committee consisting of Government officials and tsetse fly experts was organized and met at Tanga for the first time September 23-27. A report by this committee was prepared and distributed, however, the Agenda and List of Participants are included as appendices for reference. On-site consultation with this committee is a valuable contribution to the conduct of this research project and an essential liaison between agencies who must support their recommendations and our efforts.

APPENDIX "A"

AGENDA

MONDAY SEPT. 23rd

A.M. - P.M. Inspect facilities and procedures at the Tsetse Research Project headquarters in Tanga.

TUESDAY SEPT. 24th

A.M. - P.M. Reports on current status of rearing program and future plans:

1. Introduction and general status report------D. L. WILLIAMSON
2. Problems encountered in laboratory colonization of Glossina morsitans in Tanzania----------S. R. MBISE
3. Animal husbandry of goats used as laboratory hosts for G. morsitans colonies in Tanzania-------A. ISANGYA
4. Animal husbandry of rabbits used as hosts for G. morsitans colonies in Tanzania----------J. LUBUVA
5. Selection and breeding of laboratory animals as hosts for G. morsitans----------D. J. GREGORY
6. Results of rabbit-host colony experiments---E. MWAKYOLO
7. Current status of G. morsitans colonies fed on goats--------O. CHALO
8. Proposed improvements for rearing G. morsitans at Tanga--------H. BAUMGARTNER
9. Techniques for collecting and handling G. morsitans pupae in the field----------H. M. NKUNGU
10. Selection of release site and population survey plans--------D. B. GATES

WEDNESDAY SEPT. 25th

A.M. - P.M. Visit proposed release site

THURSDAY SEPT. 26th

A.M. CURRENT STATUS AND RESEARCH - requirements for assaying tsetse populations and trypanosome incidence in test site, including barrier evaluation and population suppression prior to release of sterile males.

P.M. Current status and research requirements for evaluation of efficiency of released flies, and for developing, confirming or refining methods of distribution and release.

FRIDAY SEPT. 27th

A.M. - P.M. Preparation of report and recommendations.

APPENDIX "B"

ATTENDANCE

Review Committee

Dame, D. A.	Agricultural Research Service, USDA, P.O. Box 14565, Gainesville, Fla., USA, 32604
Galun, R.	Israel Institute for Biological Research, Ness Ziona, Israel
Gao, M. K.	Tanzania Ministry of Agriculture, P.O. Box 9152, Dar es Salaam, Tanzania
Ilmolelian, L. L.	Tanzania Ministry of Agriculture, P.O. Box 9152, Dar es Salaam, Tanzania
Jordan, A. M.	Tsetse Research Laboratory, Langford, Bristol BS18 7DU, England
Konnerup, N.	Agency for International Development, Washington, D. C. USA, 20523
LeRoux, J. G.	Veterinary Laboratory, Kenya Ministry of Agriculture, P.O. Kabete, Kenya
Lindquist, D. A.	International Atomic Energy Agency, P.O. Box 590, A-1011 Vienna, Austria
Schmidt, C. H.	Agricultural Research Service, USDA, P.O. Box 5033, State University Station, Fargo, North Dakota, USA 58102.

Participants and Observers

Cornelius, J.	USAID, P.O. Box 9130, Dar es Salaam, Tanzania
Fredrickson, C.	Agency for International Development, Washington, D. C., USA, 20523
Kugler, H.	Agency for International Development, Washington, D.C., USA, 20523

Project Staff

Baumgartner, H.	IAEA/FAO	Rearing Specialist
Chalo, O.	TanGov	Asst. Field Officer, Tsetse Rearing
Gates, D. B.	ARS, USDA	Entomologist
Gregory, D. J.	AID	Veterinarian/Entomologist
Isangya, A.	TanGov	Asst. Field Officer, Veterinary
Lubuva, J.	TanGov	Asst. Field Officer, Veterinary
Mbise, S. R.	TanGov	Project Co-Manager, Field Officer, Tsetse
Mwakyolo, E.	TanGov	Asst. Field Officer, Tsetse Rearing
Nkungu, H. M.	TanGov	Asst. Field Officer, Tsetse
Williamson, D. L.	ARS, USDA	Project Manager, Entomologist