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WORKING MATERIAL

Developing product quality control for standardization of tsetse mass production

Report and Recommendations of a Consultants Group Meeting organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria, 10 – 14 June 2002.

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FOREWORD

The recent Pan-African Tsetse and Trypanosomosis Eradication Campaign (PATTEC), approved at the African Heads of State and Government Assembly in 2000, calls for the elimination of tsetse from Africa as a lasting solution to the problem of trypanosomosis. The Sterile Insect Technique (SIT), integrated with other techniques, can play a significant role in integrated area-wide tsetse fly elimination. Currently however world wide tsetse production is only 1/40 of the projected requirement in 2006. If the SIT is to play a significant role in reaching the objectives of PATTEC sterile fly production will have to be expanded rapidly, and it is essential that quality control (QC) measures suitable for the expanded production be in place.

The current rearing has only limited quality control measures. Improved QC methodology has become a top priority and will help to ensure the attainment of these production goals and improve quality of rearing, minimize production costs and generate trained QC and production staff required to successfully produce flies and monitor their quality and suitability for release.

Seven main areas are identified where improved standardized procedures are required, and specific topics within these main areas are listed. In order to achieve this, a Co-ordinated Research Project (CRP) is proposed. This report will be of use to workers in the field of tsetse rearing, and will help in determining areas of research under the proposed CRP.

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1. INTRODUCTION

1.1. The Problem

The adverse impact of trypanosomosis on human and animal health and the economy in Africa has for decades led to a variety of measures designed to control the vectors, tsetse flies, which comprise 22 separate species. For a variety of economic and environmental reasons the use of the Sterile Insect Technique (SIT) has received increasing acceptance for eliminating the last remnants of tsetse populations. The technique, having been field tested and verified is now available for eradication of already suppressed fly populations with a minimum of local adaptations. The recent Pan-African Tsetse and Trypanosomosis Eradication Campaign (PATTEC) provides a mechanism within which SIT will be one of the major components of area-wide tsetse fly elimination. Currently, worldwide tsetse fly production capacity is about 180,000 sterile males per week. The projected needs are ca. 1.5 million per week in 2004 and 3 million per week in 2006. Production expansion of these magnitudes (10X and 20X) in 2 to 4 years is unprecedented. To ensure that this expansion does not impact on the quality of sterile flies it is essential that reliable, improved quality control (QC) methods be made available.

Inherent in the expansion of production capacity are several factors that can be expected to influence attainment of the stated objectives. To realize a 20-fold production increase will require the development of several new production plants, scattered throughout Africa and nearby countries. Each plant will receive seed stock from currently existing production locations, thereby allowing for immediate propagation and expansion. A 2 year period is required for building facilities and about 1 year for establishing operational production. The minimum doubling time for tsetse colony size is 3 to 4 months, which suggests that with proper logistics and suitable rearing the minimum time required to expand 10-fold and 20-fold would be 9-12 and 15-18 months, respectively. Thus, the minimum time to reach full production of the required number of tsetse flies will be 4 years. The first large scale production facility with a capacity of 1 million sterile males per week is due to start production in 2004.

To achieve this objective it is essential the QC measures suitable for the expanded production be in place. Therefore, improved QC methodology has become a top priority. Improvements in QC methodology will not only help to ensure the attainment of these production goals, but will also improve quality of rearing, minimize production costs and generate trained QC and production staff that are mutually responsive and aware of techniques that are required to successfully produce flies and to monitor their quality and suitability for release.

1.2. Technical Support and Cooperation for QC

QC is essential for cooperating institutions to assure the quality of flies produced or purchased. Institutions that have tsetse rearing facilities include TTRI Tanzania, CIRDES Burkina Faso, FAO/IAEA Seibersdorf, KETRI Kenya, CIRAD Montpellier, France, ILRI, Nairobi, Kenya, STEP Ethiopia and LIRI Uganda and some of these will develop large scale rearing facilities. Botswana, Mali and other countries without their own facilities will purchase flies from one of these countries.

Training and continuing education will be required to maintain technical competence in QC of tsetse. Training includes *in situ* experience, exchanges among cooperating institutions, internships at key locations, fellowships at academic institution etc.

Research support for QC methods should be provided, including expertise at each location. QC personnel will help to identify and solve local production problems.

Improved communication among facilities producing and using tsetse flies will foster rapid dissemination of quality control and production information, interaction with inter-disciplinary colleagues, rapid feedback from users to producers and easy access to suppliers.

Leaders should be identified and involved in tsetse fly QC, particularly those already experienced in mass production, field evaluation and QC. These leaders will be mentors.

Experience gained in QC for tsetse will easily transfer to other pests programmes and vice versa.

1.3. Current Quality Control Methods for Tsetse Fly

The small scale rearing conducted up to the present (less than 1,000,000 females, about 100,000 sterile males per week) has not required extensive quality control monitoring. Many quality issues were handled informally by the rearing staff, who had a good feel for when the colony was performing well. As colonies expand though no individual will be able to retain an overall view.

Within the colony itself, the parameters being regularly monitored are daily mortality, fecundity and pupal emergence. Changes in daily mortality can indicate problems with the holding conditions, feeding conditions or blood contamination. Fecundity (as pupae per female per ovarian cycle) reflects the nutritional quality of the blood and holding conditions, and when expressed as pupae per initial female indicates the overall performance of the colony.

For sterile male release, the parameters monitored are sterility, mortality, fliers (the number of flies flying from the emergence box within 5 minutes), sexing error and marking efficiency.

1.4. Existing QC Methods for Other Insects Mass-Reared for SIT

Through considerable research and development, quality control procedures for the expanded production of tsetse fly can be adapted from other arthropod species that are mass produced for SIT (see appendix talks). Some quality control methods can also be derived from current tsetse fly rearing programs. Other insects from which procedures can be derived include tropical fruit flies, e.g., the Mediterranean fruit fly, Mexican fruit fly, Caribbean fruit fly, and melon fly; certain Lepidoptera, e.g., pink bollworm, codling moth, spruce budworm, and cabbage looper; and the screwworm fly. In every case, quality control procedures are used to monitor life history and behavioural traits essential for the mass production and field performance of sterile males. Life history traits typically include measurements of fertility, fecundity, rate of development, size and survival. Behaviour, such as ability to fly, find wild females, and compete with wild males, is more difficult to evaluate. Nevertheless, tests to monitor these kinds of behaviour must be developed and conducted to assure that tsetse fly males are functional after production, transport and release. As with all arthropod species mass reared for SIT, a quality control system for tsetse fly will encompass planning and administration, design and change control, quality of materials, production control, user contact and field performance, feedback and corrective action, and employee selection, training, and motivation.

2. PREVIOUS CO-ORDINATED RESEARCH PROJECTS ON TSETSE FLY

The sub-programme has run seven previous CRPs related to tsetse fly SIT. The first of these on the general application of the SIT (D4.20.01) to tsetse control in the 1970s was followed by two further CRPs on field application (D4.20.03, D4.20.04) in the 1980s and early 1990s. In addition there have been four CRPs on more specific topics, "Using radiation and isotopes to develop diets for mass rearing haematophagous insects for sterile insect release and to study disease transmission by these vectors" (D4.20.02, completed 1984), "Automation in tsetse fly mass-rearing for use in the Sterile Insect Technique" (D4.20.06, completed 2001), "Genetic applications to improve the SIT for tsetse control/eradication including population genetics" (D4.20.05, completed 2002) and "Improved attractants for enhancing the efficiency of tsetse fly suppression operations and barrier systems used in tsetse control/eradication campaigns" (D4.20.08, ongoing). Both the Genetic application and Automation CRPs have relevance to quality control issues, but neither has addressed quality control issues directly.

3. CONCLUSIONS AND RECOMMENDATIONS

3.1. Recommendations on Quality Control Areas Requiring Development

The Consultants group Meeting identified the following areas that should be address.

3.1.1. Reproductive behaviour

Quality Control tests and standards are needed to ensure the production of high quality tsetse flies. Field performance of released tsetse flies depends on reasonably complete reproductive behaviour. Tests and standards for reproductive behaviour should be developed to evaluate field performance among species and/or wild tsetse flies. Field performance of the flies depends on specific behavioural characteristics, such as sound production and detection, courtship, mate location, strain compatibility, strain competitiveness, re-mating, odour cues/attractants, and mate selection. Field cage testing of the sexual activity of laboratory reared vs wild insect strains is a prerequisite for the success in the field. The relative competitiveness of tsetse fly species and strains determines which sterile flies mate with the wild flies that are the targets for control. Knowledge of mate location and sterile fly behaviour provide the sources to direct the points of release areas.

3.1.2. Tsetse fly diet

The quality of blood is among the most important factors affecting the performance of tsetse colonies and the quantity and quality of the sterile males to be released in SIT campaigns. Blood related mortality is still a constraint in the *in vitro* system of tsetse rearing. Decontamination and QC tests are needed before the diet is given to the whole colony.

Source: Shipment of blood from Europe (Vienna) to supply Tsetse mass rearing centres in Africa is costly, fraught with delays and logistical problems and subject to quarantines restrictions and safety regulations governing the importation of labile biological products. There I a clear need to investigate suitable local blood source and reference centres in Africa.

Quality: Developing convenient acceptable procedure for collecting, processing, decontaminating, testing and storing of blood. Introducing standard blood quality test in rearing centres.

Storage and Handling: Evaluate the use of freeze-dried blood and additives for tsetse fly colony maintenance.

Additives: Investigate use of additives to improve the nutritional quality, and prevent coagulation at collection and during processing screening of each batch of collected blood.

Artificial diet: Studies to understand nutritional requirement of tsetse in relation to reproductive performances, longevity, flight, studies to find adequate diet constituents for the different species analyses to characterise the chemical, physical and microbiological aspect of the blood.

Contamination: Explore the possibility of installing a "closed system" of blood collection to further reduce contamination (bleeding under European style slaughterhouse conditions). Investigate alternative methods of decontamination (pasteurisation/ultra-high temperature procedures) and evaluate and determine their efficacy and effects on the blood quality.

Feeding regimen: Evaluate and introduce cost effective feeding regimen for the different species, e.g., time and frequency of feeding, temperature and quantity of the diet, and type of diet.

Adaptation: Studies to minimize the adaptation period of newly introduced species into laboratory conditions.

3.1.3. Irradiation of tsetse flies

The success of tsetse control programmes integrating the SIT depends on a sterilization technique for target species that produces good quality tsetse flies with the optimal combination of sterility, survival and sexual competitiveness. Sterilization of tsetse is achieved by using electromagnetic radiation from a gamma source at a dose sufficient to cause sterilization of the tsetse flies without causing undue somatic damage.

Stage of development. Too high a dose will result in undesirable somatic and genetic effects. It is vital to assess the effects of irradiation at different life-cycle stages (pupae or adults) on quality of tsetse flies. The determination of optimum physiological age for irradiation either as pupae or adults would enable the minimum radiation dose for optimum sterility and quality to be applied.

Irradiation atmosphere. Irradiation in other atmospheres (reduced oxygen, nitrogen) can improve competitiveness of the sterilized flies. Assessment of quality of flies irradiated in different atmospheres will enable programme managers to adopt the most efficient method.

Radiation exposure. The absorbed dose of radiation that is used to induce sterility is of critical importance to an SIT programme. Insects that receive too low an absorbed dose retain too much fertility for programme purposes. Too high an absorbed dose will result in insects that do not compete well against wild flies in the field. However, 100% sterility is not essential.

Dosimetry. A standardized and practical method is required for calibrating irradiators for use in SIT programmes. By adopting a standardized dosimetry system, uniformity in irradiation can be achieved at the different production centres.

3.1.4. Field release studies

Assessment of tsetse fly quality in the field before and during control operations is essential for determining optimum release numbers and frequencies. Many of these fitness parameters are subject to the influence of handling and processes that occur during the production,

packaging and distribution phases. These include, but are not limited to, vector capacity, flight ability, nutritional status, and stage and age at the time of release. Methods to predict the status of these parameters may be possible through specific pre-release monitoring. Studies in outdoor cages may be used to detect obvious and subtle behavioural changes and deficiencies.

Confirmation of the reliability of this fitness monitoring can be obtained by conducting specific field release studies. Monitoring specific effects and impacts of the released flies can only be accomplished in the field. Specific field studies will be required to determine appropriate field sampling techniques to reveal changes in distribution patterns and fertility of indigenous fly populations and mating behaviour of the released flies.

Marking flies prior to release provides a reliable method for such assessments. Discovery of a genetic marker that does not impact fly quality would simplify this procedure.

3.1.5. Colony maintenance

Strain management and compatibility. There is a need to survey all species targeted for SIT for their genetic diversity both in the field and those already in different rearing centres. This will save time used to adapt or start new colonies from wild flies and in some cases will help to identify which colonies are compatible. The importance of this is illustrated by the salivary gland hypertrophy virus (SGHV) in the *G. pallidipes* Ethiopian strain which may cause a delay in the mass production of the Ethiopian strain. In this case, a compatible strain should be reared whilst the issue of SGHV is being investigated.

Colonization of species: The early colonization of all species targeted for SIT is important so that all key parameters for tsetse rearing are studied and documented. This should include performance in captivity, the mating behaviour, ratios, strain compatibilities and sex separation. Also colonization of tsetse species should include a relatively large number of founders for better heterozygosity.

Salivary gland hypertrophy: The salivary gland hypertrophy virus is an issue for concern especially with the *G. pallidipes* Ethiopian strain. It is important that a study should be carried out to answer some of the questions like the cause of infection, transmission and how it can be controlled. The investigation should include all species targeted for SIT and all species currently being reared by different centres which will take part in rearing species for SIT application, so that preventive measures are taken in order to prevent any delay for mass rearing programmes.

Sex separation: It is now clear that the system to separate sexes without chilling is in place. Emphasis should be made so that all centres which will take part in rearing tsetse for SIT application replace the old system of sex separation by self stocking of production cages (SSPC). This is one of the key issues which will eliminate chilling and ensure that flies produced are vigorous (Opiyo et al., 2000, Zdarek & Denlinger, 1995).

Feeding equipment and materials: The feeding equipment and materials must be thoroughly sterilized, heating mats (heating source) and feeding membranes controlled at the right temperature.

Mortality checks: There are two types of mortality recognized in tsetse flies, with or without blood in the gut. Increased mortality with blood in the gut indicates a problem with feeding or holding conditions. Mortality should be checked to assure normal feeding and holding conditions. To reduce fly handling during the mortality check, a decision by tsetse rearing

personnel should be made to determine whether the source of mortality is due to the blood or starvation.

3.1.6. Facilities, equipment, and materials for QC

It will be necessary to have adequate facilities, equipment, materials and staff available for the QC for expanded production. A suitably sized QC facility is required that has temperature, humidity and light controlled areas divided for fly holding, emergence etc. with bio-security, with separate space for each species handled, contained within the rearing facility. A separate pathology area is also required for blood testing etc. The office space for the QC should be adjacent to the production office to ensure continuous interaction between QC and production. The QC area will need its own support services, storage etc. and suitable locations needs to be identified for field tests.

The Head of QC must be of equal status with the production management. The QC staff should receive appropriate specific training, leading to a recognized qualification. The staff should be dedicated to QC, and have a background in rearing so that they fully understand the issues involved, and their experience should be refreshed frequently. Continuing education and training are essential, and exchange of experience between facilities should be encouraged.

Specialized equipment will be required for laboratory and field QC tests. For ease of exchange and comparison of results, the QC operations should utilize standardized equipment specifications in addition to standard procedures.

3.1.7. Harmonization of QC Methods

It is essential that standardized QC methods be developed and used for all tsetse fly production facilities and field operations, so that data can be compared and problems solved quickly. Standardized data format and management protocols will facilitate data exchange. The manual of uniform QC methods will be based on a manual of standard operating procedures used at every location. Eventually, production may be under an international certification and review process along the lines of ISO-9000. This kind of certification may be required for transporting tsetse flies between countries to assure the identity and purity of shipments. Harmonization of QC will also facilitate exchanges of personnel among locations engaged in tsetse fly production and SIT. It will simplify training and evaluation of personnel, and periodic program reviews. Similarly, health and safety QC can be instituted uniformly across locations. Harmonization of QC methods will enhance communication among personnel and contribute significantly to their motivation.

3.2. Conclusions

The Consultants Group Meeting examined the current status of knowledge and various QC options for use in producing and releasing tsetse fly and other arthropods. Their conclusions were:

1. Standardized tsetse rearing and field assessment quality control protocols will be essential for the successful expansion of the sterile insect technique for tsetse control to the scale envisaged in the PATTEC proposal.

2. A Co-ordinated Research Project focused specifically on QC to address these issues would be the most effective way to achieve these objectives and is therefore worthwhile and justified.

3. Unlike previous CRPs, which addressed the general application of SIT for tsetse control, odour attractants and genetics, a new CRP should focus on the improvement and harmonization of current QC methodology and the development of QC methods suitable for rapid expansion and long term tsetse production capability and field assessment.

A draft proposal for a new Co-ordinated Research Project can be found in Annex 1.

APPENDIX 1 AGENDA OF THE MEETING

Monday 10 June			
08:30	09:00	Arrival at VIC, obtain Ground Passes	
09:00	09:30	Welcome / Administration	A. G. Parker
09:30	10:30	The PATTEC initiative and implications for tsetse rearing	U. Feldmann
10:30	*10:45*	*Coffee break*	
10:45	11:15	The Research Contract System	J. Reed
11:15	*12:00*	*Transport to Seibersdorf*	
12:00	*13:30*	*Lunch*	
13:30	16:00	Visit to the Entomology Unit, Seibersdorf	
16:00	*17:00*	*Return to VIC*	
Tuesday 11 June			
08:30	09:00	Recent developments in tsetse rearing at Seibersdorf	A. G. Parker
09:00	09:30	Current research on QC issues at Seibersdorf	G. Mutika
09:30	10:30	Tsetse production issues in CIRDES	I. Kabore
10:30	*10:45*	*Coffee break*	
10:45	11:45	Tsetse production issues in Tanga	I. Malele
11:45	*13:00*	*Lunch*	
13:00	13:30	Process and product quality control in fruit flies	D. Orozco Davila
13:30	14:30	Process and product quality control in Lepidoptera	N. C. Leppla
14:30	15:30	Quality control issues in tsetse	D. A. Dame
15:30	*15:45*	*Coffee break*	
15:45	17:00	Discussion	
Wednesday 12 June			
08:30	10:30	General discussion - Identification of problems	(N. C. Leppla)
10:30	*10:45*	*Coffee break*	
10:45	12:30	General discussion - areas for research and development	
12:30	*14:00*	*Lunch*	
14:00	15:30	General discussion - areas for research and development	
15:30	*15:45*	*Coffee break*	
15:45	17:30	General discussion - areas for research and development	
Thursday 13 June			
08:30	10:30	*Divide into groups for drafting* Drafting of report	(N. C. Leppla)
10:30	*10:45*	*Coffee break*	
10:45	12:30	Drafting of report	
12:30	*14:00*	*Lunch*	
14:00	15:30	Drafting of report	
15:30	*15:45*	*Coffee break*	
15:45	17:30	Drafting of report	
Friday 14 June			
08:30	10:30	Drafting of report	(N. C. Leppla)
10:30	*10:45*	*Coffee break*	
10:45	12:30	Compiling of sections, preparation of Logical Framework	
12:30	*14:00*	*Lunch*	
14:00	15:30	Presentation of report	
15:30	*15:45*	*Coffee break*	
15:45	17:30	Presentation of report	

APPENDIX 2 PRESENTATIONS

U. Feldmann

1

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History of international cooperation on Tsetse / Tryps

- 1975: FAO starts a special action programme action against tsetse and trypanosomosis
- efforts on R&D and control remain uncoordinated
- control efforts result in no alleviation of the tsetse and trypanosomosis problem
- funding declined
- early 1990's: Donors start a sub-regional tsetse eradication project ("goalposts" are later changed) basically without the involvement / consultation with FAO and other international organisations and FAO's special action programme is eventually discontinued

3

the Programme Against African Trypanosomiasis (PAAT)

a concerted effort
to clarify and solve
the problem of
African Trypanosomosis

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Objective of PAAT

To solve the trypanosomosis problem
within the broader context of
food security,
human health,
rural development
and sustainable agriculture

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Initial PAAT policy

- effective methods exist to control both human and animal trypanosomosis in most agro-ecological zones
- main problems are logistical
  - correct application of drugs and vector control
  - sustainability at acceptable cost
- instead of vector and disease eradication: intervention in selected areas using an integrated disease management approach
  (live with but reduce problem below economic threshold)

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- FAO General Assembly approves PAAT as replacement of previous Panel of Experts
- WHO World Health Assembly approves PAAT
- OAU's InterAfrican Bureau of Animal Resources (IBAR) collaborate in PAAT to harmonise efforts and resources
- IAEA also collaborate to harmonise efforts and resources but faces difficulties: resulting from specific MS requests, IAEA Board approved projects aim at tsetse eradication (initially not supported by PAAT)
- IAEA's conditional collaboration favoured a review of the PAAT promoted approaches and led to the acceptance of the area-wide approach and the option to create tsetse fly free zones

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At meetings, etc., informal discussions about PAAT reflected:

- Usefulness of the concept and the issues addressed, but also the
- Perceived domination of issues by non-Africans:
  Donors –> policy and project implementation issues
  European Universities –> research and method development

In response PAAT initiated the transfer of its "Policy and Implementation Module" to OAU-IBAR

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Chronology of Developments (1)

- South and Central America, 1980s and 90s: Staff of Moscamed projects advance Medfly SIT through establishing a regional network for exchange of relevant developments in mass production and field operations;
- 1996 / 97: With the completion of the Zanzibar and anticipated expansion of similar integrated campaigns on Mainland Africa, the idea is developed to establish a similar African network for advancing tsetse SIT
- Conference at Kluang, Malaysia, May 1998: First discussions on the establishment of such a network;
- Gainesville, FL, USA, May 1999: Interregional Training Course: Group of African participants discuss the establishment of forum for technical information exchange on tsetse SIT

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Chronology of Developments (2)

- October 1999: golden jubilee meeting of OAU's International Scientific Council for Trypanosomiasis Research and Control (ISCTRC). Recommendation to African governments to give highest priority to tsetse / trypanosomosis
- May 2000: Upon invitation of the Ethiopian Government and under the auspices of OAU, African entomologists and veterinarians establish the Pan-African SIT Forum
- 10-12 July 2000: 36th Summit of African Heads of State and Government passes a "Decision on Proposal for Eradication of Tsetse Flies on the African Continent" [AHG/Dec.156 (XXXVI)]

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Chronology of Developments (3)

- Sep. + Nov.'00: FAO, IAEA, WHO and other collaborating partners welcome the "summit decision" as a historic declaration.

To generate tsetse-free zones, various steps are needed, involving disease management and tsetse suppression prior to rendering an area tsetse free.

Integrated area-wide campaigns will likely involve a SIT component for final vector eradication.

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Chronology of Developments (4)

- Nairobi, December 2000: A task force of African Experts establishes the Pan-African Tsetse and Trypanosomosis Eradication Campaign (PATTEC)

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Chronology of Developments (5)

- Lusaka, Zambia, July 2001: 37th Heads of African States and Governments approve the PATTEC action plan.
- Vienna, Austria, September 2001: IAEA General Conference pass a resolution in favour of PATTEC
- Ouagadougou, Burkina Faso, October 2001: Official Launching of PATTEC at the occasion of the 26th ISCTRC
- Roma, Italy, November 2001: FAO General Conference pass a resolution in favour of PATTEC

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Initial Priorities for PATTEC

1. Awareness generation
   - National: inclusion of the tsetse/"trypa" issue in the PRSPs
   - International: recognition of the tsetse/trypa problem as a "root" problem preventing development (ECOWOC and other fora)
   - Donors' support
2. Success stories needed
   - 3 priority areas for intervention
     - Ethiopian Valley systems
     - Cotton belt or moist savannah in West- and Central Africa, starting with Burkina-Faso/Mali
     - Botswana
   - In addition: opportunity areas identified (KEN, UGA, URT, ...)
3. Regional Tsetse Factory/ies

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Agreed Criteria for Priority Intervention Areas (1)

Agreed in PAAT / PATTEC Harmonisation meeting 2/3 May 02

- Severity of the impact of the T&T problem
- Desire / need for intervention by local communities and national governments
- Opportunity to support poverty reduction, increase food security and maximise socio-economic returns through enhanced SARD, such as
  a) Expansion and intensification of mixed farming;
  b) Improved subsistence farming and / or production of cash crops;
  c) Land use and tenure as components of sustainability;
  d) Sustainable and environmentally appropriate utilisation of natural resources.

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Agreed Criteria for Priority Intervention Areas (2)

- Factors contributing to increased feasibility and early success of project activities and sustainable outcomes, such as
  - a) Activities phased and initial objectives achievable within 5 – 7 years of a programme / project cycle;
  - b) Natural barriers;
  - c) Possibility for artificial confinement;
  - d) Favourable agro-ecological production trends;
  - e) Favourable climatic variations and trends;
  - f) Commitment and involvement of local authorities and communities;
  - g) Existence of local technical and logistical support;
  - h) Existence of ongoing agricultural development project that identifies T&T as major constraint.

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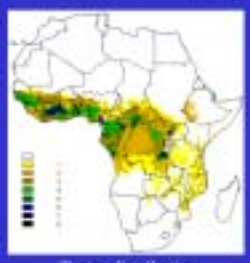
Agreed Criteria for Priority Intervention Areas (3)

Climatic zones in Africa



Tsetse distribution
a > 80% of the infested area
max. 1 to 3 tsetse species

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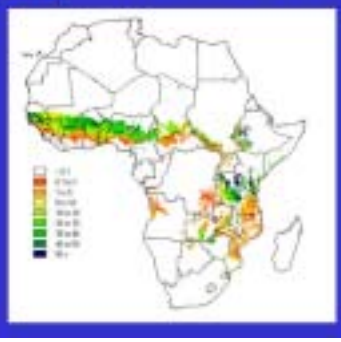
Agreed Criteria for Priority Intervention Areas (3)



Livestock potential

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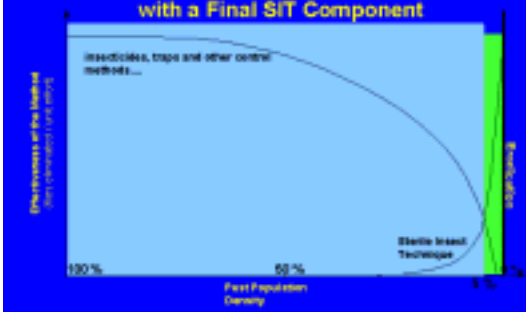
Area-Wide Integrated Pest Management with a Final SIT Component



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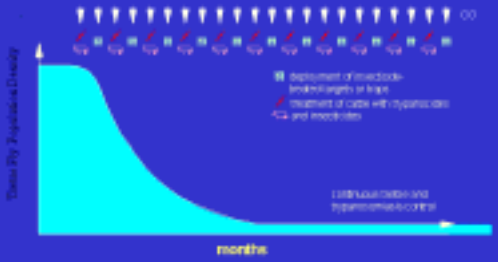
Integrated Tsetse Intervention for Permanent Control



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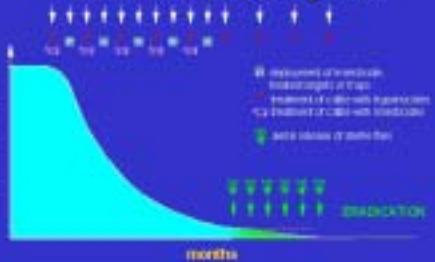
Integrated Tsetse Intervention with a Final SIT Component



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Creation of Tsetse-Free Zones based on areawide approaches

- 'Rolling-up' approach
  continuous expansion of fly free area
- Tsetse "islands"
  - Zanzibar, Mafia
  - Ethiopian valley systems
  - Okavango delta
- Tsetse "peninsulas"
  - Lake Victoria area
- confined tsetse "pockets"
  removed on by one

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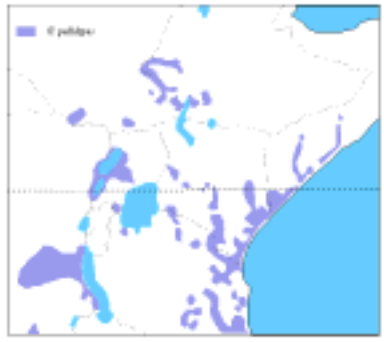
Population-Genetic Mapping

- Satellite imagery – tsetse risk prediction
  - Additional field work
  - Transects and identification of sampling points
- Trapping at 30-50 km interval along transects
- at least 50 flies per trapping location
  - Head and thorax for DNA examinations
  - gonadal tissues, except for spermathecae and uterine content, -> screening for gonadal symbionts (*Wolbachia*)
  - remaining part of abdomen eventually for blood meal analysis

Population-genetic mapping based on tsetse prediction maps (map for *G. palpalis* [ERGO / TALA])

In addition: mark-release-recapture studies

23



Approximate distribution of *Glossina palpalis* in Eastern Africa (after ORSTOM/CIRAD, 1988)

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Approximate distribution of *Glossina fuscipes fuscipes* in Eastern Africa (after ORSTOM/CIRAD, 1988)

25

Approximate distribution of *Glossina morsitans centralis* in Southern Africa (after ORSTOM/CIRAD, 1988)

26

Need for Sterile Males
upcoming challenges

- Currently projects produce own males. In future customers will purchase flies (quality criteria for payments)
- Efficient use of males. Need to determine the numbers of males really needed in each area (operational field research with some modelling)
- Production of sterile males with certain desired characteristics (for example refractoriness of strain to trypanosome infection)
- Production of more males in sub-regional tsetse rearing centres

27

Need for Sterile Males
current rearing capacity (col. fem.)

Country	capacity	current size
TTRI Tanga, Tanzania	1 million	95,000
CIRDES, Bobo D., BKF	250,000	150,000
FAO/IAEA Seibersdorf	200,000	100,000
KETRI, Mostp., ILRI, etc.	200,000	10,000
Kaliti, ETH	150,000	25,000
Total	**1,850,000**	**380,000**
		(21% of capacity)

28

Need for Sterile Males
rearing needed 2004

Country	st'm / km²	km²	col. fem.
MLI	80	2,500	2,000,000
ETH	80	8,500	6,400,000
BOT	30	12,000	3,600,000
URT	75	1,500?	1,125,000
KEN	75	300	225,000
UGA	75	1,500	1,125,000
Total		**25,800**	**14,475,000**

Current capacity is 13% of anticipated 2004 needs

29

Need for Sterile Males
rearing needed 2006

Country	st'm / km²	km²	col. fem.
BKF/MLI	80	10,000	8,000,000
ETH	80	15,000	12,000,000
BOT	200	300	600,000
URT	75	4,000	3,000,000
KEN	75	2,500	1,875,000
UGA	75	5,000+	3,750,000
Total		**36,800**	**29,225,000**

Current capacity is 6.5% of anticipated 2006 needs

30

A. Parker

Following Carol Calkins' division, Quality control can be separated into Production, Process and Product Quality Control.

1. Production QC (is there a better name for this? – to me this does not clearly identify this element) covers the inputs to the rearing process, which in the case of tsetse means principally diet. The only other item directly involved in the rearing are membranes and cages.
2. Process QC covers the actual protocol of rearing, environmental conditions, handling procedures, feeding procedures, irradiation etc., and perhaps the release protocol as well. Although strictly this is a separate issue, because of the way tsetse SIT is currently set up it should logically also be included here.
3. Product QC is perhaps the most obvious, and concerns the quality of the product – that is how well the sterile male flies compete with the wild males. Absolute quality is not important though, it is cost effectiveness that matters. Factors here include flight ability, mate finding, competition with wild flies, insemination potential etc.

The only significant item under Production QC is blood. Blood is currently collected at the abattoir, defibrinated, irradiated to reduce bacterial contamination, and then it is checked for residual bacterial load and bio-assayed for nutritional quality. The blood collection and handling procedures should be subject to Quality Control measures.

Currently two potential improvements are being investigated:

1. The first it to use pasteurization to replace irradiation. The need to irradiate the blood is a serious constrain on tsetse production, as the necessary irradiators are not generally available. The IAEA supplies irradiators to projects, but this is only possible where the necessary radiation safety legislation is in place. In many countries this is not ready, and this is a potential source of serious delays to projects.
2. The second is some form of synthetic diet. Ideally this should be shelf storable at (tropical) room temperature, waterless (to be light to transport), commercially available

and of equal nutritional quality to defibrinated blood. A PhD student is just starting work on some of these aspects.

Further work could also be done on alternatives to defibrination (particularly citration) and other additives to improve the nutritional quality. It has been know for many years that a mixture of porcine and bovine blood is better than either alone, so presumably each is lacking or short in some essential component.

Product quality control, the performance and cost effectiveness of the sterile male flies is being covered by Gratian.

The issues in rearing relating to product quality include the genetics of the colony flies and physical development.

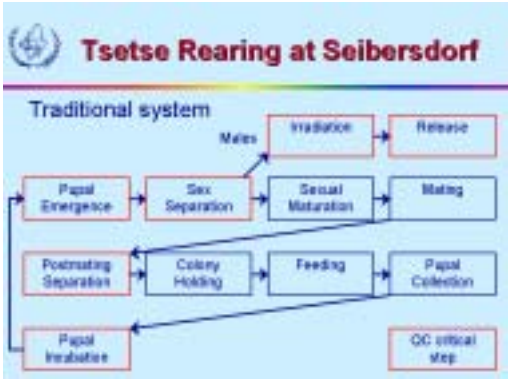
Bringing any insect in to colonization involves enormous selection pressure on the first few generations to adapt to the artificial conditions. The consequent "bottle-neck", and subsequent partial recovery in heterozygosity due to the accumulation of random mutations over the generations has been recorded for several species, but the significance of these findings in not clear, and little has been done on tsetse. We have just initiated an effort to catalogue the tsetse in colonization around the world, and to collect samples periodically from these colonies for genetic analysis to follow changes over time. We are also collecting genetic information about wild populations, and it may eventually be possible to draw meaningful conclusions from comparisons of these data. Currently the behavioural work is the only meaningful comparison that can be made.

Physical development, in particular flight ability, is a critical issue for cost effectiveness, and work is urgently needed on this.

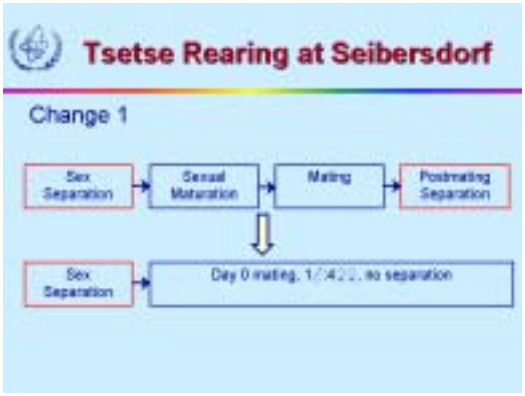
Process quality control probably offers the easiest area in which to devise QC standards. This includes environmental conditions, blood handling and feeding, pupal handling, pupal emergence and sex separation and chilling.

- Environmental conditions are defined for the holding of tsetse colonies, and only adequate monitoring of these parameters is required.
- Blood handling and feeding is one of the most critical stages. Poor blood can destroy a colony in a matter of days, and require years for recovery due to the low reproductive rate. Any programme reliant on a single source of sterile flies would be effectively destroyed by a catastrophic colony loss like this.

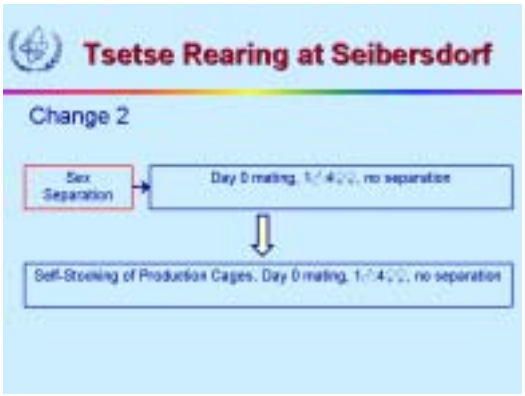
- Pupal handling and emergence is more a cost effectiveness issue. Suitable handling and emergence procedures can greatly reduce production costs, improve emergence rates, and provide males for sterilization with a low proportion of residual females.
- Chilling for any reason is known to be detrimental, so any procedure to reduce or remove chilling is advantageous. A number of developments in this field have been made in Seibersdorf and elsewhere.
- Finally irradiation is another important area. Irradiation inevitable impacts fly quality, and these negative effects must be minimised. Work on this is currently continuing at Seibersdorf under Gratian Mutika.



The traditional holding system was very labour intensive, and involves a number of quality critical steps. Over the last 7 years many advances have been made both in Seibersdorf and Tanga.



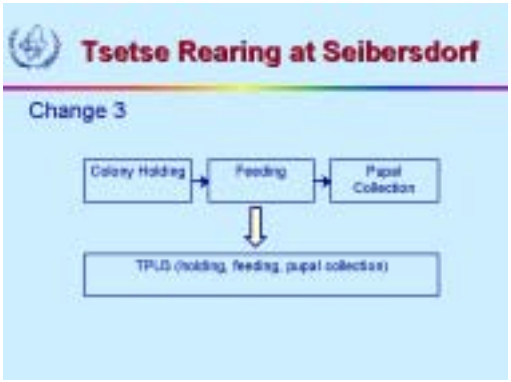
The first improvement was the removal of post mating chilling for separation. This was accomplished by reducing the number of males used for mating from the original 1:1 or 1:2 to a ration of 1:4 (depending on species) male per female. At this reduced ratio the disturbance to the females causes les harm than the chilling to separate them would. Further it was observed that the traditional aging of flies before mating as not necessary – flies can be caged together from emergence and will mate once mature.



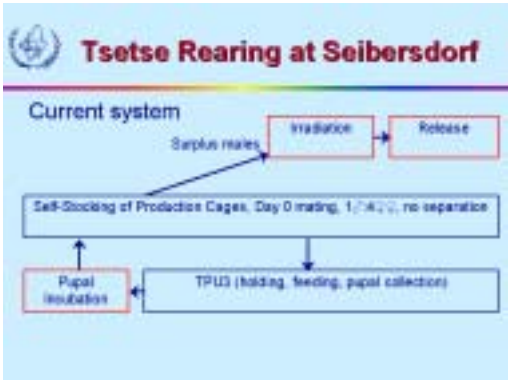
This logically lead to a system whereby the flies emerge direct into the production cages at an appropriate ratio. Females always start to emerge from the pupae before males, and by manipulating the temperature it is possible to cause almost all females to emerge in the first 48 hours. Newly emerged flies crawly upwards towards light, and can therefore be made to crawl into a cage. With an appropriate small net size on the top they can not crawl out of the top of the cage, and once the wings are expanded they can no longer pass through the lower, larger mesh.

Depending on species, either no males, or very few males, have emerged by this time. For species where no males emerge a portion of male only pupae from a previous emergence can be added to batches of pupae where the females are just about to emerge; where males start to emerge earlier, by careful timing the necessary number of males can be emerged into the cage. This is not so accurate, but ratios between 1:3 and 1:6 can be tolerated without

compromise to colony production. Whichever system is used, the remaining mostly male pupae can then be emerged for irradiation and release.



The third main development recently is to remove the handling of individual cages of flies. Under the old trolley system cages had to be manually placed on a feeding membrane and later returned to the trolley. This regular movement disturbs the flies, using up energy and reducing fecundity. The new TPU3 system holds the fly cages still on a rack, and the blood is brought to the flies.



The current TPU3 system can be summaries like this.

The reduction in labour greatly reduces the room for human error in the rearing process. The remaining steps that may be addressed are pupal holding, and the processing of male flies for sterilizing.

Work at Seibersdorf will centre on the following in the near future:

1. Containerized rearing – a prefabricated rearing system in shipping containers that was relocatable would solve some of the current problems of shortage of rearing facilities, and the siting of facilities.
2. Pasteurization of blood as an alternative to irradiation
3. An easy to store, commercially available, cheap synthetic diet
4. Hybrid sterility
5. Investigation of the salivary gland hyperplasia virus, and development of an assay for it. This virus severely affects the fecundity of susceptible strains of *Glossina pallidipes*.
6. Investigation of the mechanism whereby male tsetse locate female flies. The conventional story that this occurs in the "following swarm" does not fully answer the problem, as male tsetse are capable of locating females in the absence of a host, even when the female is stationary some distance from the male (2 - 3 meters in a field cage). The possibility that ultrasound is involved will be investigated.
7. A centralized recording and control system for the environmental conditions in the insectaries will be investigated. Any such system must be robust and immune to power supply problems.

G. Mutika

The quality of mass reared tsetse flies that were released during previous sterile insect technique (SIT) programmes were assessed in various ways.

Mark-release-recapture studies to determine survival, dispersal and flight ability.

Mating tests in production cages or Perspex tubes in the laboratory - limited space and choice.

Flight ability and mortality checks were also carried out prior to and after the release flight - sample boxes retained for this purpose.

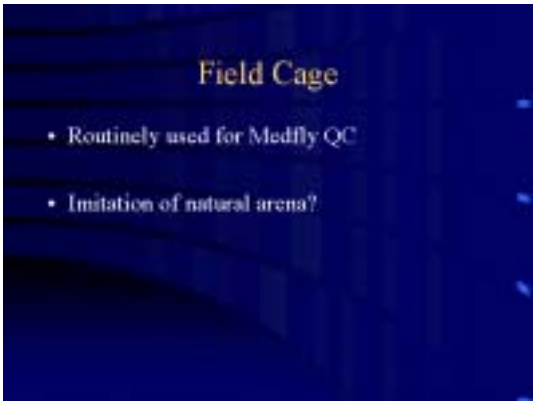
Efforts are being made to improve quality control through the use of field cages to assess several parameters that include mating behaviour. It is critical that the sterile males released in an SIT programme can equally (or better) compete for mating opportunities against wild males to ensure successful transfer of sterile sperm to wild females.

Mass reared sterile males should also transfer competitive sperm.

Field cage assessment of quality of mass reared flies was attempted in the natural environment in the Zambezi Valley, Zimbabwe (Dame *et. al*. 1969) - there was no direct observation of fly activity.

Other quality control work is focussing on development of the adult chilled release system. We are exploring an alternative to use of cardboard boxes when releasing sterile flies from an aircraft.

Typical set up of a field cage.



Field cages are routinely used in assessment of quality of mass reared fruit flies

There are several indices that can be derived from results of field cage studies

Although some of the indices are particular to fruit fly studies there is a possibility of some of them being modified/adapted for application to other insects including tsetse flies.

The use of field cages tries to imitate the natural environment - located in the field in conditions where the flies naturally occur.

Offers opportunity for direct observation of several aspects including mating behaviour

The initial work on assessment of male quality using a field cage at Seibersdorf was carried using *Glossina pallidipes* (Mutika *et al*., 2001)

For details of methods and results please refer to the above paper. The field cages at Seibersdorf are erected inside a green house to allow work to continue during the cold months.

Propensity of mating - the overall proportion of released females that mate during the defined observation period. Represents the overall mating activity of the flies under the given environmental conditions and is used to assess suitability of the conditions and the flies for the test.

Relative mating index - the number of pairs of one treatment group as a proportion of the total number of mating. Values range from 0 to +1.

Relative mating performance - the difference between the numbers of matings of the two types of males (e.g. total unirradiated pairs minus total irradiated pairs) as a proportion of the total number of matings. Values range from -1 to +1.

Field cage observation of mating behaviour has since been carried out with four more tsetse fly species at Seibersdorf

A greater proportion of irradiated flies mated than unirradiated - indication of good, competitive flies at the sterilization dose used.

The mating compatibility of *G. pallidipes* strains from Ethiopia and Uganda was assessed. The Uganda strain has been reared for over 25 years while rearing of the Ethiopia strain started six years ago.

Male tsetse flies from the Uganda strain readily mated with female tsetse flies from the Uganda strain.

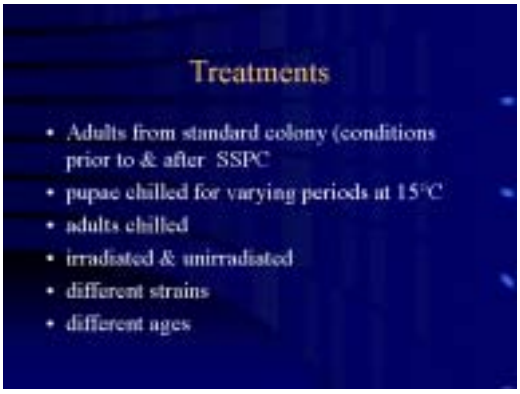
Most of the male flies from the Ethiopia strain that did not mate had salivary gland hypertrophy

The mating compatibility of *G. morsitans centralis* strains from Tanzania and Botswana was assessed.

The Tanzania strain has been reared for several generations since the mid-1970's while the rearing of the Botswana strain started about two years ago.

Male tsetse flies from the Tanzania strain readily mated with female tsetse flies from the Botswana strain.

Competitive mating tests among different ages of males of *G. fuscipes fuscipes, G. brevipalpis* and *G. palpalis palpalis* were also carried out in the field cages.



Assessments in the field cages were carried out on flies treated in various ways

- Adults that emerged under Seibersdorf standard colony conditions (24°C, 80% r.h., 16 hours dark: 8 hours light)
- Adults emerged under self stocking of production cages (SSPC)
- Adults emerged from pupae chilled for varying periods at 15°C (irradiated and unirradiated).

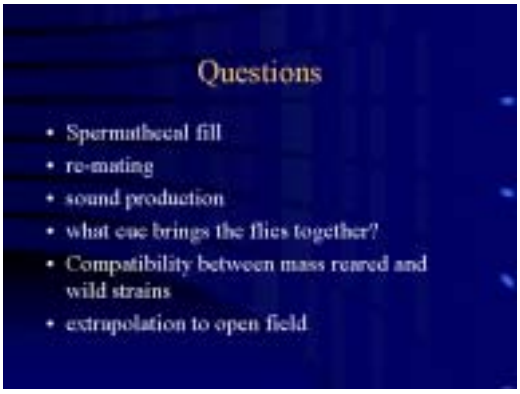
Eclosion can be held back for up to 72 hours when male pupae are chilled at 15°C without significant effect on mating ability, survival and sperm transfer. There is also the added benefit of synchronization of emergence and flush emergence (over 60% of males emerge within 24 hours after removal from 15°C and transfer to 26.5°C)

Adults chilled at 4°C and 7°C for various periods of time. Survival and mating behaviour. The longer the flies (adults) are chilled the greater the mortality effect, chilled adult males also transfer lower volume of sperm and accessory gland fluid (Mutika *et al*., 2002)

Adults irradiated in air and in nitrogen with unirradiated controls. Chilling of adults after irradiation for 6 hours at 4°C significantly lowered survival and transfer of sperm.

Different strains (previous slide)

Different ages of males. The older the males the greater the proportion mating in competitive tests. Used males from two to fourteen days old.



Can we improve on determination of spermathecal fill, actual counting of individual sperm, volumetric determination or this subjective quantification is suitable for our work?

Is there sperm precedence in the event of re-mating, especially important for SIT in females that first mate with irradiated males. Other workers content that there is no negative impact on an SIT programme due to re-mating.

In *G. pallidipes* and *G. morsitans centralis* there were occasions when engagement of genitalia was preceded by a sound audible to the human ear, does it have any significance in mating behaviour?

How does the male locate a female when the female has not moved? What cue enables correct identification of mate?

Is there good mating compatibility between mass reared and wild flies and also between males from different geographical regions? Other workers have shown that different geographical strains are compatible but data not available for all economically important species.

How representative in the open field is information gathered in a field cage?

I. Kabore

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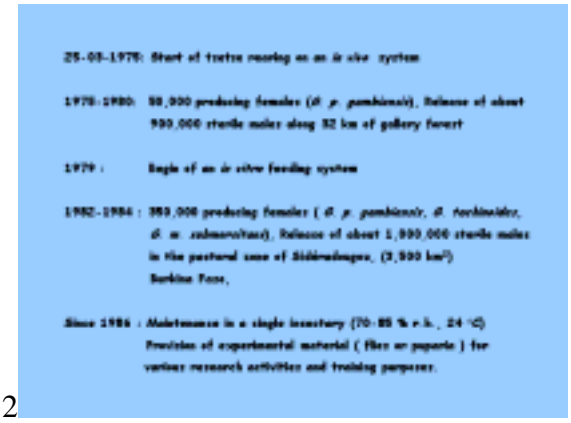

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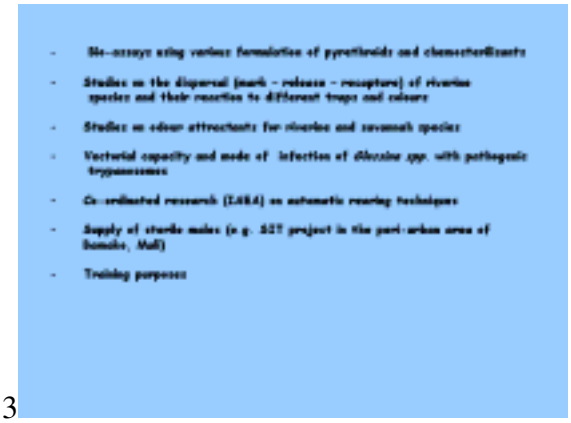

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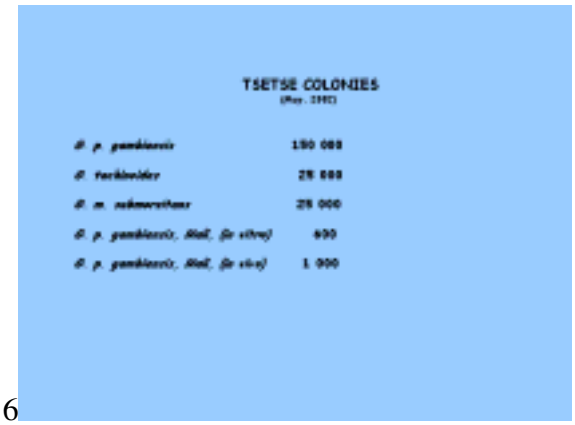

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Counting pupae based on volumetric extraction (*G. p. gambiensis*, *G. tachinoides*, *G. m. submorsitans*)

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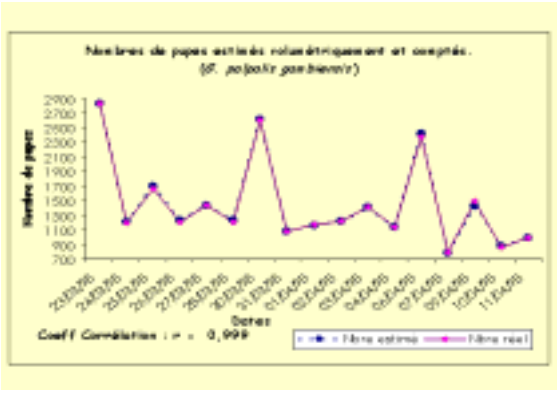

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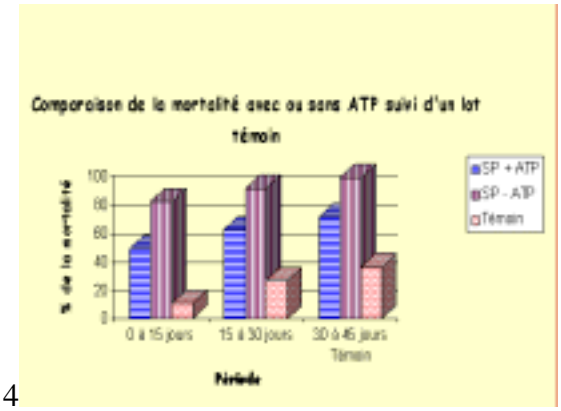

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Emergence of flies directly into production cages (*G. p. gambiensis*, *G. tachinoides*, *G. m. submorsitans*)

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18 

I Malele

Improvements on rearing procedures and its benefits to fly production for SIT application

Malele, I. I, Msangi, A. R., Kitwika, W., Byamungu, M. & N. E Kiwia
Tsetse & Trypanosomiasis Research Institute, P. O. Box 1026 TANGA TANZANIA

INTRODUCTION

The status of tsetse mass rearing relative to that which has been developed for other SIT targeted pest, is a major constrain for the wider application of this technique for tsetse. Being K selected and obligate haematophagous, the reproduction rate is very low and in nature they are adapted to feed on sterile blood obtained directly from blood vessels of host animals. So, cleanliness in the lab and *in vitro* feeding equipment is important for tsetse survival and reproduction.

Also, fly handling procedures have to be reduced in number and scope to enable a more economic production of flies to be accomplished. Traditional tsetse rearing procedures were described by Nash *et al*., (1968) and Tarimo *et al*., (1984). However in order to have excess males for release on Zanzibar, several improvements had to be done to improve the rearing conditions in order to have a steady producing colony for production of quality sterilized males. Years of rearing at TTRI, plus the collaboration with the Tsetse group at Seibersdorf has demonstrated that many procedures can be simplified without compromising fly production and quality. Several of these procedures were adopted for *G. austeni* colony and enabled the increase in colony size, increased pupae production which led to the increase in excess males for release. The end result was the successful eradication of *G. austeni* in Unguja Island (Msangi *et al*, 2000). Factors behind the success in tsetse rearing in Tanga are reviewed below.

Improvement of rearing facilities

Insectaries were renovated and new equipment fitted. Sensors to detect changes in temperature and relative humidity were fitted. These were automatically switched on and off for optimal rearing environment (22- 24^{o}C and 70 - 80% RH). Water troughs around the insectaries and fly holding trolleys were fitted to prevent predators (ants).

Other important additions were the installation of a standby generator for a constant supply of electricity, construction of water bore hole and a water tank for a continual supply of water for sanitation in lab and rearing equipment like feeding trays, membranes, cages etc.

Two walk in freezers with a 10,000 litre capacity for blood storage to ensure smooth availability of fly diet and a clean air station UV Hood for checking blood contamination, computers for data processing, communication equipment, heating mats with thermostats to provide a stable temperature, timers to synchronise the operation of heating mats and ovens, electrical protection equipment, digital thermometers for monitoring chiller temperatures were all purchased and fitted in the labs. Another boost to the rearing activities was the installation of a new gamma source with bigger irradiation chamber for irradiating blood for feeding the fly colony especially when the colony was bigger, and during sterilization of males destined for release.

REARING

To have a steady growing tsetse colony, it was important to make sure that the colony daily mortality was kept below 1%, and fecundity (P/F/10d) was above 0.5. To increase the eclosion rate, pupae vials were placed on top of moistened sponges after 25 days if incubation so as to improve humidity.

Mating ratio of 1 male: 5 females & no separation of sexes after mating

Having demonstrated that there was no need to mature the sexes separately before mating and that when fewer males are put in the production cages together with females they could be left together without the need for separation without compromising performance, colony maintenance of *G. austeni* was revised and day zero mating was introduced (Malele & Parker, 1999). The benefits from this adoption was the elimination of second chill to separate sexes from mating and this had a good impact on producing females. This method eliminated a need for twice handling of production cages when separating sexes from mating, eliminated the need for separate cages to handle maturing males and females, and also because of the ratio of 1 male: 5 females, excess males were recovered for release.

Bulk irradiation of males

About 3000 - 7000 males were bulk irradiated at 120 Gy in a chilled thermos flask (0.5 litre), and this saved labour and time used to irradiate flies, and minimized excess handling of flies.

Diet of flies

Flies were fed in vitro on whole defibrinated bovine blood through silicone membrane warmed to 37^{o}C by heating mats. Possible bacterial contamination of blood was reduced by irradiation with gamma rays (^{60}Co). Contamination was checked by mixing blood with a sterile medium and monitored for 24 - 48 hours for any bacterial growth. If there were more than five bacterial colonies, the blood was discarded, but if less than five colonies, then the blood was irradiated again before use. A small number of flies were fed for 25 days to assess the quality of blood before being made available to the whole colony. Continuous bacterial screening reduced the risk of feeding flies with unsuitable blood, and the colony was spared from unnecessary mortality.

Samorin fed males

To reduce the transmission of trypanosomosis by released males, sterilized males destined for release were twice fed with trypanocide treated blood as described by Moloo & Kamunya (1987).

Quality assessment of sterilized males destined for release

Boxes with flies destined for release were picked randomly and parameters like sexing error, mortality, induced sterility, marking, fliers and non fliers were scored. Results showed that flies were of good quality. On average, more than 93% of all flies were able to fly, only 2.6% died and sexing error was 0.66%. Induced sterility and marking was 100% (Kitwika *et al*., 1997).

Fly monitoring

For every flight, a sample of the flies for release was taken in Zanzibar to assess its quality. The data were compared with those taken in Tanga just before the shipment and indicated that the release operation did not seriously affect the quality of flies. Fly mortality increased by only 2% and proportion of non - fliers increased to 1.6% (Saleh *et al*., 1997).

CURRENT SITUATION

The evaluation of *G. austeni* performance held on new TPU version is continuing at TTRI.

CONCLUSION

The key factor is to ensure that flies produced are of good quality both for colony growth and sterilized males for release. Efforts should be made to minimize handling of flies in all stages of production.

The eradication of *G. austeni* in Unguja Island is an indication that flies produced in the labs were able to compete against wild males.

WHAT NEEDS TO BE DONE

More research to reduce handling of flies. Excessive manual handling is detrimental to flies.

SSPC: If the remaining pupae are predominantly males can we explore the possibility of irradiating pupae and then the emerged males be left to mature in the lab for some time in order to develop flight muscles, Samorin fed and then released?. Langley (1970), points that males should be released in the field after they have completed their musculature development. The age of released *G. austeni* males in Zanzibar was 3 – 6 days old (Kitwika *et al.,* 1997).

Irradiation dose for females: Can it be revisited again to see if we can make use of hydrocarbons found in female tsetse which elicit copulatory responses to males (Wall & Langley, 1993). This can be used to lure wild males to mate with sterilized females, so as to increase the chances of sterilized flies competing with wild flies.

ACKNOWLEDGEMENT

IAEA, URT, Tsetse group at Seibersdorf, Technicians at TTRI

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D. Orozco Davila

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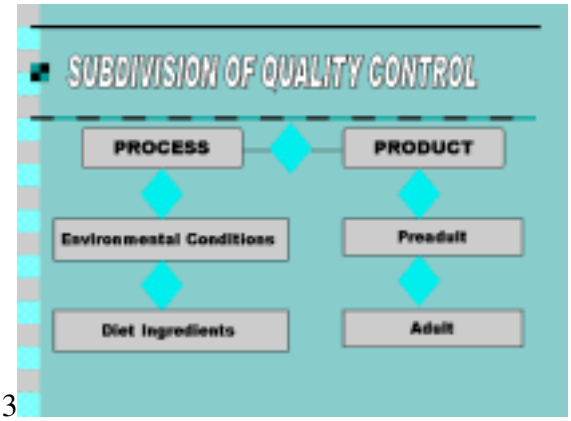

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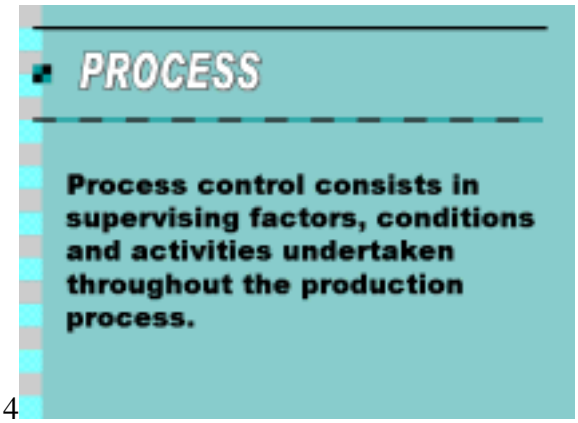

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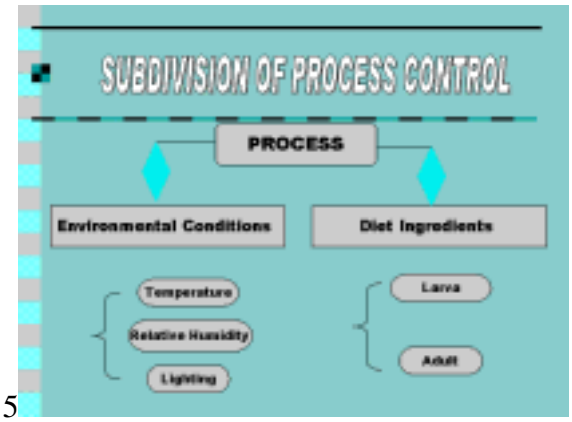

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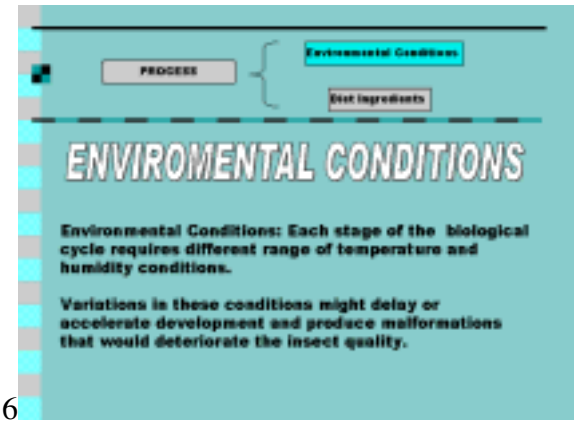

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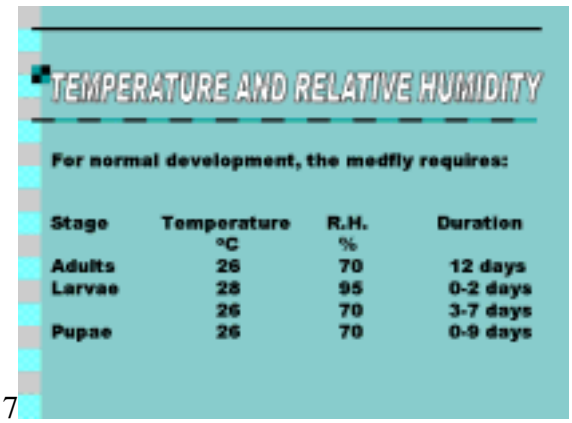

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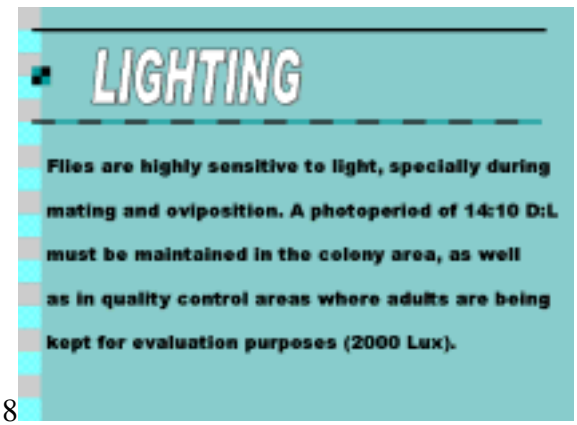

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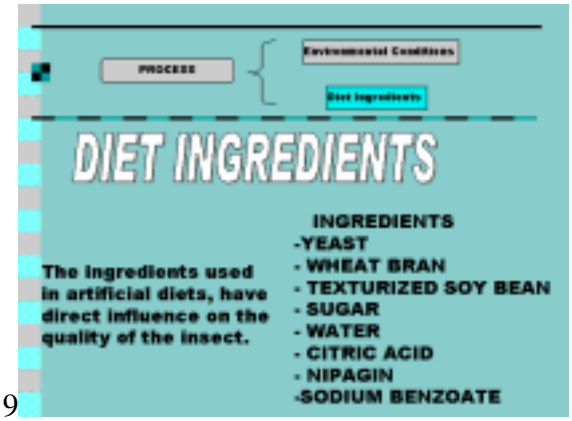
PROCESS
Environmental Conditions
Diet Ingredients
DIET INGREDIENTS
The ingredients used in artificial diets, have direct influence on the quality of the insect.
INGREDIENTS
-YEAST
- WHEAT BRAN
- TEXTURIZED SOY BEAN
- SUGAR
- WATER
- CITRIC ACID
- NIPAGIN
-SODIUM BENZOATE

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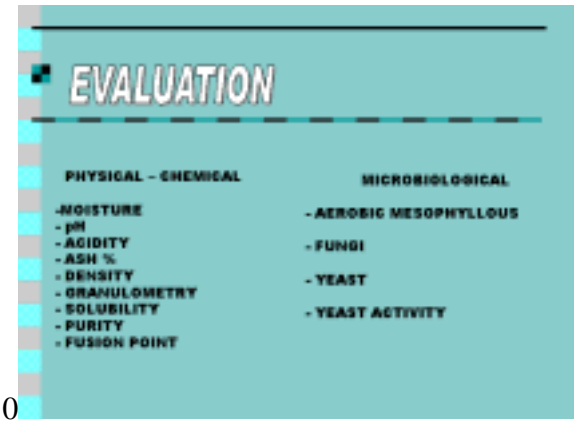
EVALUATION
PHYSICAL – CHEMICAL
-MOISTURE
- pH
- ACIDITY
- ASH %
- DENSITY
- GRANULOMETRY
- SOLUBILITY
- PURITY
- FUSION POINT
MICROBIOLOGICAL
- AEROBIC MESOPHYLLOUS
- FUNGI
- YEAST
- YEAST ACTIVITY

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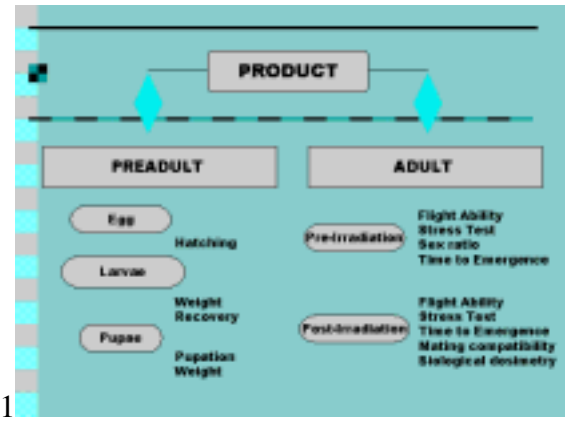
PRODUCT
PREADULT
ADULT
Egg
Hatching
Larvae
Weight Recovery
Pupae
Pupation Weight
Pre-irradiation
Flight Ability
Stress Test
Sex ratio
Time to Emergence
Post-irradiation
Flight Ability
Stress Test
Time to Emergence
Mating compatibility
Biological dosimetry

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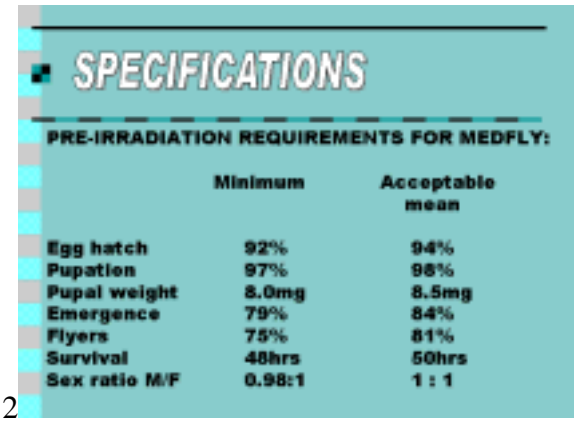
SPECIFICATIONS
PRE-IRRADIATION REQUIREMENTS FOR MEDFLY:
Minimum
Acceptable mean
Egg hatch 92% 94%
Pupation 97% 98%
Pupal weight 8.0mg 8.5mg
Emergence 79% 84%
Flyers 75% 81%
Survival 48hrs 50hrs
Sex ratio M/F 0.98:1 1 : 1

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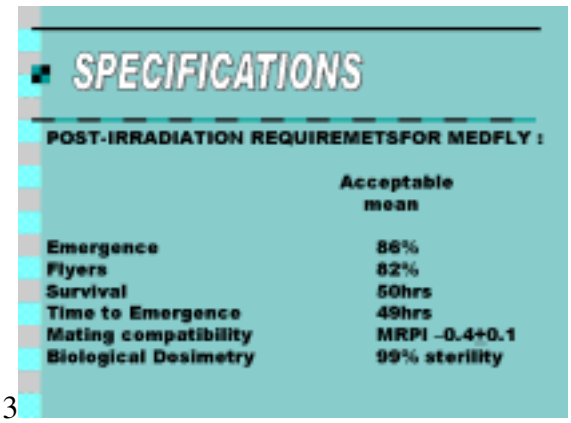
SPECIFICATIONS
POST-IRRADIATION REQUIREMETSFOR MEDFLY :
Acceptable mean
Emergence 86%
Flyers 82%
Survival 50hrs
Time to Emergence 49hrs
Mating compatibility MRPI –0.4+0.1
Biological Dosimetry 99% sterility

13

N. C. Leppla

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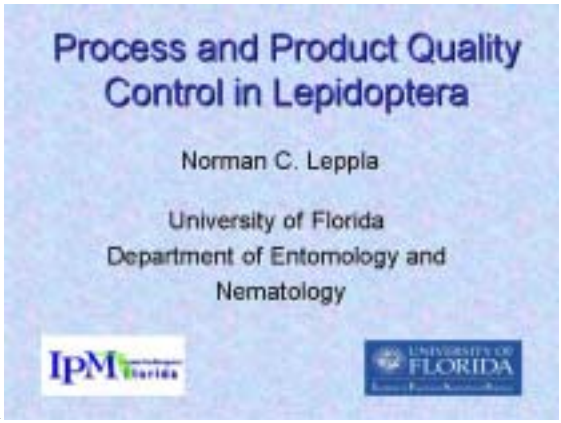

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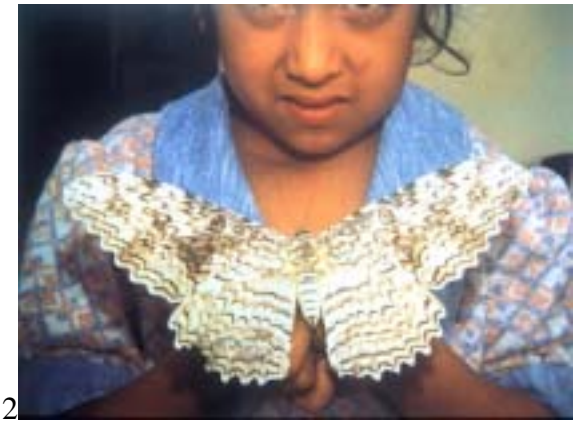

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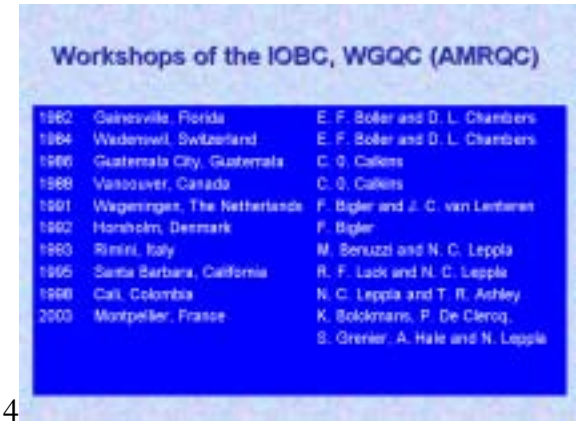

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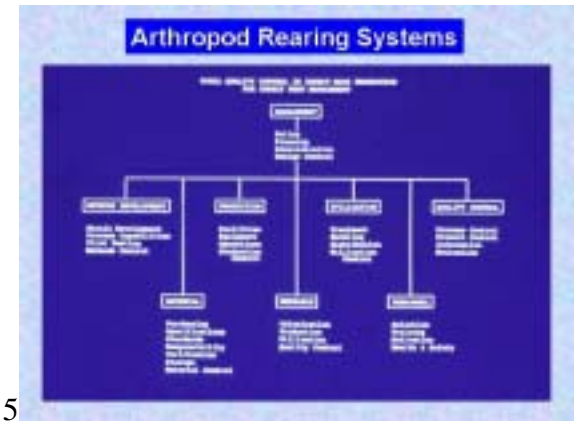

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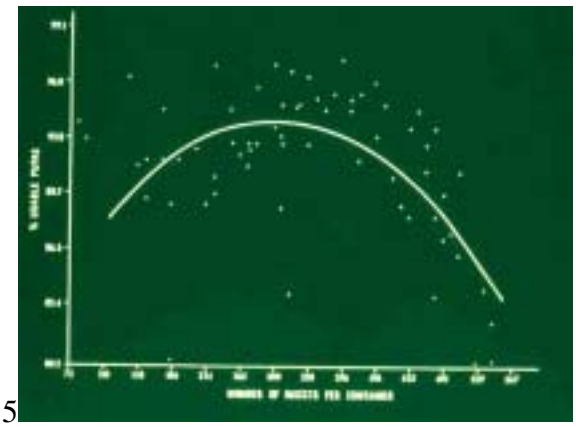
15 

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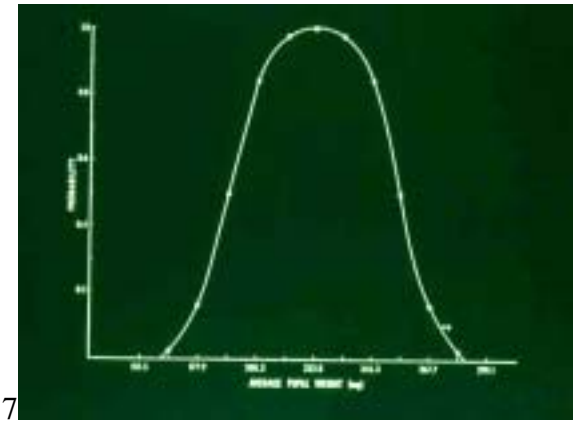

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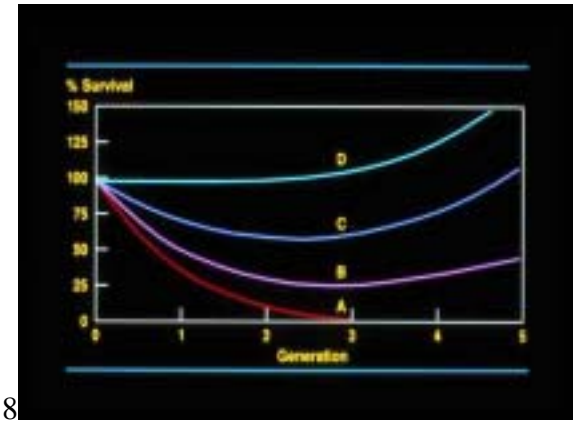

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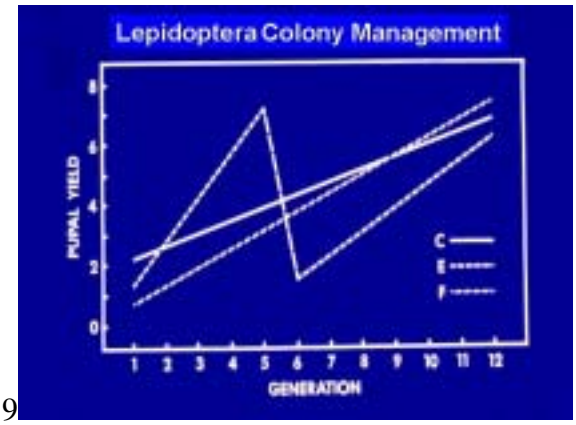

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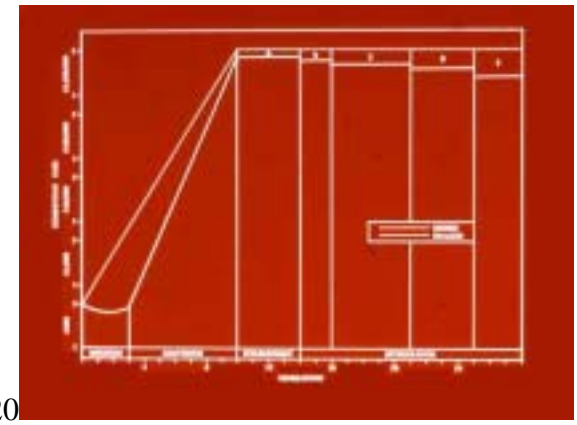

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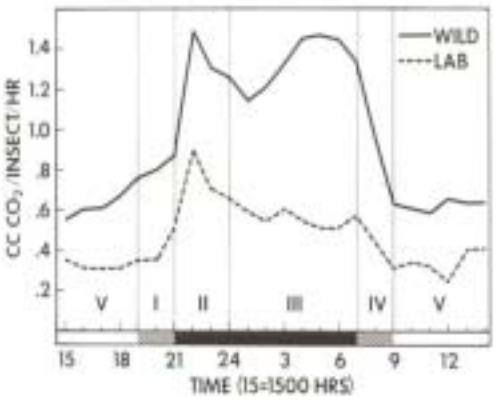

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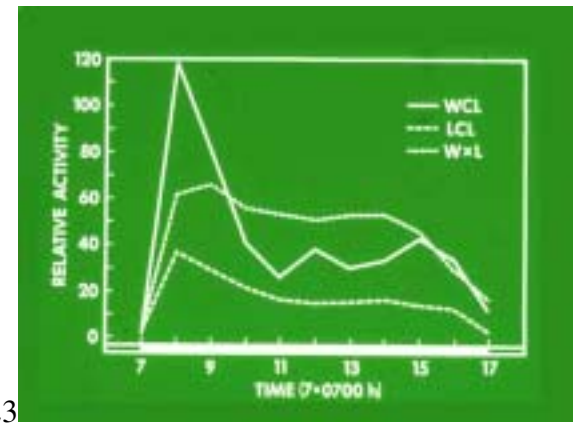

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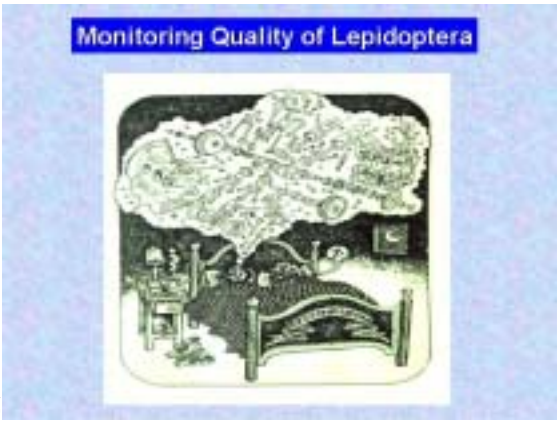

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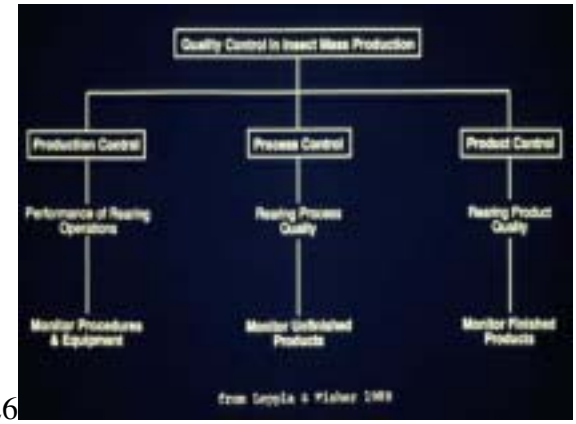

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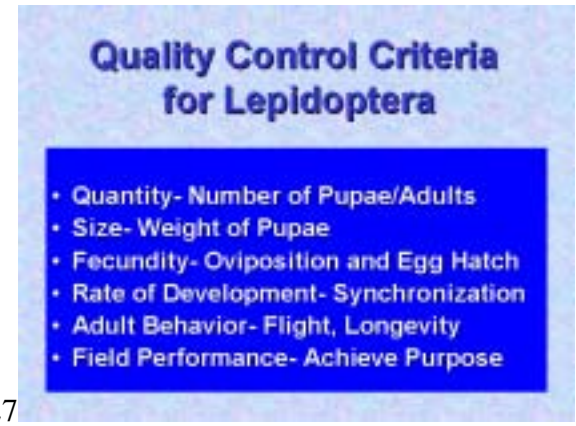

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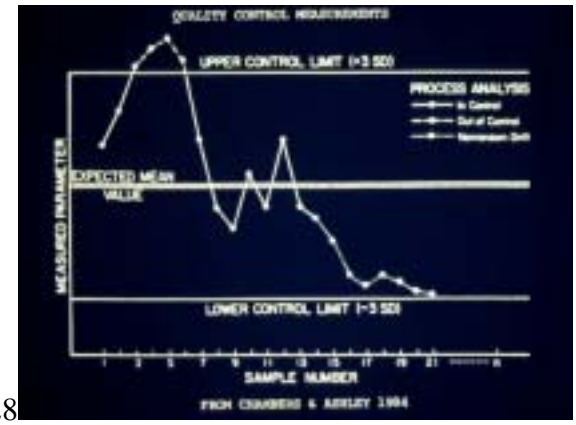

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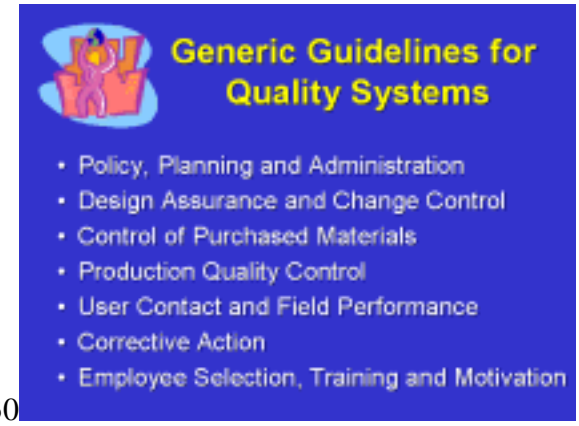


31

D. A. Dame

Issues of Fitness and Hardiness in Sterile Tsetse Flies

David A. Dame, Entomological Services
Gainesville, Florida

I. INTRODUCTION

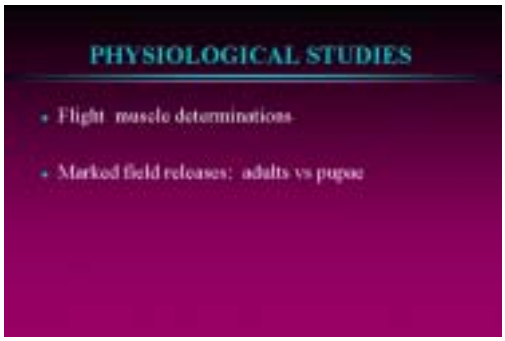
The impact of released sterile tsetse flies is affected by biological factors resulting from ambient conditions and from handling encountered during rearing. Behavioural traits as well as fitness and survival characteristics can be influenced at this time. Ecological conditions such as host availability, vegetation patterns, topography and the distribution, density and rate of increase (RI) of the natural population may also influence the impact of sterile tsetse flies. This presentation addresses the behaviour in the field of released tsetse flies in connection with ecological and pre-release handling factors.

Some of these factors can be manipulated to the advantage of the control strategist. For example, administering blood meals containing trypanocides can forestall subsequent infection and transmission of these pathogens. Selection between the late pupal stage and early or later adult stage for release can enhance the mating success of sterile **flies**, depending on species and local conditions. Careful implementation of these and other factors may enhance efficacy of the released flies and at the same time contribute to enormous cost savings. Failure to take advantage of these and other options could magnify program costs and create undesirable logistical problems.

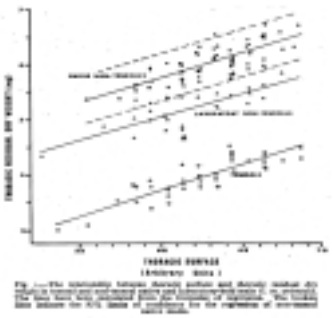
For example, proper relative humidity, cage stocking or temperature during rearing and handling helps to maximise the numbers of flies produced and their hardiness. Suitable irradiation procedures, such as appropriate timing and provision of a protective nitrogen atmosphere enhances fitness and survival. With nitrogen irradiation can yield dominant lethality yet avoid risk of severe somatic damage (Curtis and Langley, 1972). Reduced radiation exposure can further protect fly quality and still cause enough sterility (e.g., >85%, which may cause F_1 sterility in the next generation if any progeny survive) to **enhance** population control. But when such precautions are overlooked, numerical and quality losses may force the use of increased release rates or reduced area coverage in order to maintain program continuity. In the long run this increases not only programme costs but also the numbers of potential vectors that are released. Avoidance of adverse conditions through effective quality control procedures is usually cost-effective.

II. STUDIES WITH *Glossina morsitans morsitans*

Initial releases. Early field studies provide examples of handling effects. Feasibility studies for the use of SIT with tsetse were initiated in 1967 with *Glossina morsitans morsitans* on an island in Lake Kariba in Zimbabwe. Adult flies that had emerged from field-collected puparia were chemosterilized by tarsal contact and released at pre-selected sites throughout the island. To reduce the natural population to levels suitable for sterile insect releases two aerial insecticide applications were conducted, one 28 days and the other 1 day before starting the releases. The subsequent release rate of sterile males was designed to provide a 1:1 ratio, or higher, of sterile: fertile indigenous flies. The resulting captures of sterile flies in routine fly rounds was a meagre 12% of the captures of indigenous wild flies (indicating a ratio of only 1 sterile : 8 fertile indigenous flies) and the *G m. morsitans* population began to recover. After 6 months the release programme was discontinued.



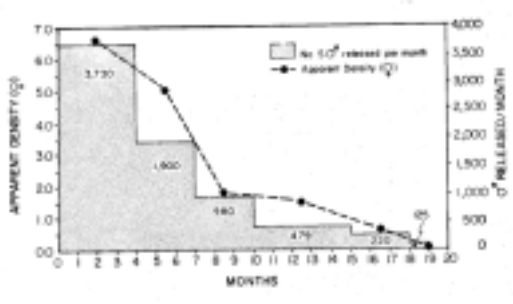
Physiological Studies. Failure of the released flies to control the natural population led to studies to determine the reason(s) for their poor performance (Dame, Birkenmeyer and Bursell, 1969).



First, a comparison of flight muscle size was conducted with wild caught non-teneral flies and newly emerged and 7-day old fed flies that had been held in cages similar to those used in the release trial. After 7 days the size of the flight muscles of only 17% of the caged flies was similar to that of the wild flies (Fig.1, **slide 6**)).

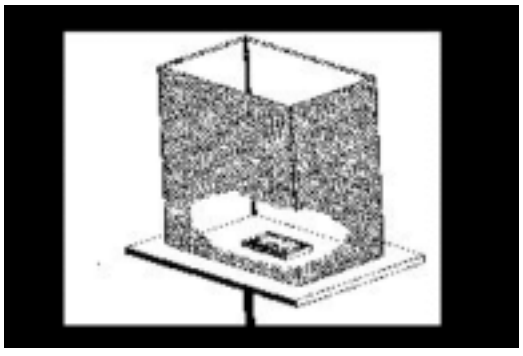
Marked puparial and adult releases. The observed 12% incidence of sterile flies in the release trial (compared to the indigenous flies) fairly closely matched the observed 17% that had normal flight musculature after 7 days (2 blood meals) in cages. To determine if these observations **of low quality** might be related, releases of marked flies were conducted in normal fly habitat where routine fly rounds were being conducted weekly. Flies that had been caged were marked and released from a single point near the centre of the fly round grid. From the same point flies were marked as they emerged from pupae and allowed to

disperse. The results of two replications showed no differences in mean time to recovery or in mean distance from release to recovery (Table 1; **slide 7**). But the caged fly recovery was only 17% of that of wild flies recaptured during the same time period, compared to 96% for the flies that had dispersed directly from the puparium. This was the third incidence of performance below 20% of normal for flies held in cages after emergence, providing reason to suspect that the phenomena were in some way associated.



Adult releases at an increased ratio. Following these findings releases were initiated on another island where the *G. m. morsitans* male population was estimated to be about 600 per sq km. The releases were conducted at a rate calculated to be 5-fold greater than the natural population to offset the observed 80% or greater deficiency observed in the flies. Each quarter the release rate was halved, as projected in Knipling's original models for tsetse fly SIT control (Dame and Schmidt, 1970). After 18 months the island's fly population had been eliminated (Fig. 2; **slide 8**). Since one would theoretically anticipate elimination in about 12 months at a ratio of 3 sterile : 1 fertile indigenous, it was concluded that the competitiveness in the field of the released male in this circumstance was probably about 20%.

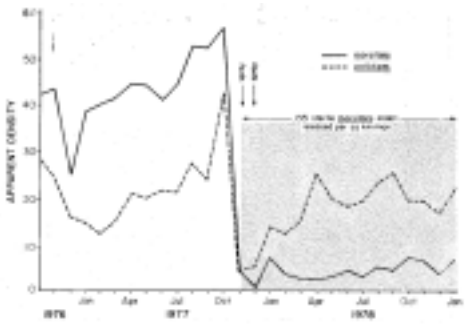
Puparial release. As a result of the above findings releases were re-instituted in the original test site, but this time with flies that emerged in the field from chemosterilized puparia. Once again aerial insecticide application was utilized to reduce the indigenous population. The *G. m. morsitans* population was further reduced to very low levels by the sterile males, whereas *G. pallidipes* (control) recovered (Dame & Schmidt, 1970; Dame, Lowe and Williamson, 1981). Because of political events this study was interrupted before completion. However, the observations from the 2 releases, the marked fly field study, and the physiological study of flight muscle development were sufficient demonstration of feasibility of SIT for tsetse that funding was continued for further field study after relocation.



Tanga program. Having resettled the project in 1972 and then completed construction of 3 insectaries for *in vivo* propagation of *G. m. morsitans* , new studies were initiated near the Tanzania coast at Tanga. With the assistance of a large, trained staff donated by the Tanzania Ministry of Agriculture and project-saving backup support from IAEA-Vienna, the largest tsetse colony to date was established. As *in vitro* rearing was not yet available, production on goats was selected as the method of choice. Quality control parameters for this production facility are outlined in Williamson, Baumgartner et al (1983a). Funds that would otherwise have been used to support rearing research were diverted (ca. $250,000) to IAEA to support *in vitro* research and the establishment of a backup *in vitro* colony.

Puparia were held at 24 C until about 52% emergence had occurred (mostly females and enough males for colony replenishment). The remaining puparia were then held at 4 C for up to 4 days and then irradiated in a nitrogen atmosphere, which provided protection from severe somatic damage (Curtis and Langley, 1972) that otherwise would reduce puparial

survival and adult fitness, and helped by suppressing eclosion during irradiation. They were then chilled at 8 C for the 3-6 hr transport to the field plot at Mkwaja ranch. Upon arrival they were dispatched to designated release sites (Fig. 3; **slide 9**) where they were placed under sand containing Day-Glo powder for automatic marking at emergence (different colours each 2nd week, repeated after the 14th week, for monitoring purposes). Synchronous eclosion was completed within 60 minutes. Each station received a delivery every two weeks, with numbers prorated from the total release to match the observed density of the indigenous population.



Mkwaja release. The 195 sq. km research plot was encompassed by a 1 km wide cleared barrier reinforced with residual DDT application on the boles of trees most likely to attract tsetse. Two aerial applications provided prior suppression of the two major species present. **Immediately** following the first application sterile releases were initiated, and continued for a year at the rate of 135 sterile males per sq. km (Williamson, D.L. et al. 1983b) The results of the program are shown in Fig. 4 **(slide 10)**. Complete elimination of the target population was not achieved. A residual population of ca. 10% persisted following the initial impact of the released flies in the first few months, although *G. pallidipes* (control) recovered completely.

To determine why the remaining 10% of the *G. m. morsitans* population was not eliminated, a test was conducted to check on the porosity of the 1 km barrier around the test plot. Over 50,000 marked sterile males were released outside the barrier and their entry into the test plot was monitored by fly rounds that were being routinely conducted to monitor the population. A total of 18 of flies released outside the barrier was captured in the test plot. The fly round capture efficiency estimate (0.00225, based on an average of 278 flies normally captured from the pre-release population estimated to be ca. 123,000) was used to calculate that ca. 8000 (18/0.00225) marked flies had actually crossed the barrier. This number of immigrants (16%) was determined to be sufficient to explain the presence of the residual population.

Figure 5 **(slide 12)** shows the calculations used to reach the conclusion that the residual population was due to immigration. Immigrant females would have been fully fertile. Females within the test plot would have been 50% fertile because of the observed 1:1 ratio of sterile:fertile males observed within the plot. Both would have been influenced by the average 1.15 (RI) observed throughout the year. Calculations based on the accumulated data projected the RI to be 0.93 within the test plot, compared to the observed RI of 0.96 concurrently outside the test plot. This finding suggested that the sterile males were operating at full efficiency (close to 100% competitiveness).

III. CUMULATIVE FINDINGS WITH STERILE TSETSE FLIES

Reported estimates of effective sterile to indigenous male ratios in SIT trials and programmes conducted throughout Africa are shown in Fig. 6 (**Slide 13**; Dame et al 1981, Vreysen et al 2000, Cuisance et al 1986, Politzar et al. 1980, Williamson, Dame et al. 1983b). *Glossina morsitans morsitans* was involved in Zimbabwe and Tanzania (Mkwaja) releases, the riverine species and *G. m. submorsitans* in West Africa and *G. austeni* in Zanzibar. Each location differed greatly in habitat. The *G. m. morsitans* releases were manually conducted in savannah grassland and mopane woodland (**1 sterile:1 wild, 5 sterile:1 wild**), the riverine releases were manually conducted along river margins and associated forested habitat (**10 sterile:1 wild**), and *G. austeni* (**10-20 sterile :1 wild**) was aerially conducted in mixed habitat that included very dense vegetation. Mode of release and habitat strongly influence the probability of encounters between released males and indigenous females. It may be impossible to determine efficacy directly and these estimates of required ratios are not exact. However, the findings reveal a wide range of possible outcomes in terms of overflooding ratios required. Managers of tsetse SIT programmes need to be aware of and take full advantage of the manageable factors that influence fly fitness in order to better and more cost-effectively cope with factors over which they have no control. Considerations of fitness, numbers available for release, and the logistics of holding adult flies prior to release are key components of this equation.

IV. CONCLUSIONS

Even if this ratio interpretation is unwarranted, the results of the several programmes tend to confirm that handling methods in rearing, irradiation and distribution processes have a significant impact on the quality of the released sterile flies. For example, pre-release blood meals are useful for reducing the probability of pathogen transmission by the released fly, but may involve a severe trade-off in the sense that fewer flies are released because of mortality during the holding phase and possible loss in flight ability.

Where it is feasible, there seems to be adequate justification to use nitrogen to protect irradiated flies from excessive somatic damage. The need for 100% in released tsetse is questionable. With their low reproductive potential, it is possible that 85% - 90% male sterility would be adequate to obtain elimination faster than with 100% sterility because of somatic damage resulting from high sterilization levels. This could be even faster if the F_1 sterility induced then contributed to population reduction in subsequent generations.

However, with the limited experimental evidence at our disposal it is obvious that these matters need to be resolved, perhaps for each species being considered for SIT. In the meantime, SIT managers need to be cognizant of the range of positive and negative impacts of these factors on released flies.

The development of QC methodology for expanded tsetse fly production should address these parameters that are so intimately related to the effectiveness of the released fly in the field.

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CONTRIBUTORS TO DRAFTING AND REVIEW

Dame, D. A.	USA
Feldmann, U.	International Atomic Energy Agency
Kabore, I.	Centre International de Recherche et Développement sur l'Élevage en Zone Subhumide, Burkina Faso
Leppla, N. C.	Department of Entomology and Nematology, University of Florida, USA
Hendrichs, J.	International Atomic Energy Agency
Malele, I.	Tsetse & Trypanosomiasis Research Institute, United Republic of Tanzania
Mutika, G.	International Atomic Energy Agency
Orozco Davila, D.	Methods Development and Field Evaluation, Programa MOSCAMED, Mexico
Parker, A. G.	International Atomic Energy Agency

ANNEX 1 PROPOSAL FOR A CO-ORDINATED RESEARCH PROJECT

1. TITLE:

Improved and Harmonized Quality Control for Expanded Tsetse Production, Sterilization and Field Application (Project E.4.02, Activity 1)

2. BACKGROUND SITUATION ANALYSIS (RATIONALE/PROBLEM DEFINITION)

2.1. The Problem

The adverse impact of trypanosomosis on human and animal health and the economy in Africa has for decades led to a variety of measures designed to control the vectors, tsetse flies, comprising about 6-7 species of major economic importance. For a variety of economic and environmental reasons the use of the Sterile Insect Technique (SIT) has received increasing acceptance for eliminating the last remnants of already suppressed tsetse populations. The technique, having been field tested and verified, is now available for establishment of tsetse-free areas with a minimum of local adaptations. The recent Pan-African Tsetse and Trypanosomosis Eradication Campaign (PATTEC) provides a mechanism within which SIT will be one of the major components of areawide tsetse fly elimination. Currently, worldwide tsetse fly production capacity is about 180,000 sterile males per week. The projected needs are ca. 1.5 million per week in 2004 and 3 million per week in 2006. Production expansion of these magnitudes (10X and 40X) in 2 to 4 years is unprecedented. To ensure that this expansion does not impact on the quality of sterile flies it is essential that reliable, improved quality control (QC) methods be made available.

Inherent in the expansion of production capacity are several factors that can be expected to influence attainment of the stated objectives. To realize a 40-fold production increase will require the development of several new production plants, scattered throughout Africa and nearby countries. Each plant will receive seed stock from currently existing production locations, thereby allowing for immediate propagation and expansion. A 2-year period is required for building facilities and about 1 year for establishing operational production. The minimum doubling time for tsetse colony size is 3 to 4 months, which suggests that with proper logistics and suitable rearing the minimum time required to expand 10-fold and 40-fold would be 9-12 and 15-18 months, respectively. Thus, the minimum time to reach full production of the required number of tsetse flies will be 4 years. The first large-scale production facility with a capacity of 1 million sterile males per week is due to start production in 2004.

To achieve this objective it is essential the QC measures suitable for the expanded production be in place. Therefore, improved QC methodology has become a top priority. Improvements in QC methodology will not only help to ensure the attainment of these production goals, but will also improve quality of rearing, minimize production costs and generate trained QC and production staffs that are mutually responsive and aware of techniques that are required to successfully produce flies and to monitor their quality and suitability for release.

2.2. Technical Support and Cooperation for QC

QC is essential for cooperating institutions to assure the quality of flies produced or purchased. Institutions that have tsetse rearing facilities include TTRI Tanzania, CIRDES Burkina Faso, FAO/IAEA Seibersdorf, KETRI Kenya, CIRAD Montpellier, ILRI Kenya,

STEP Ethiopia and LIRI Uganda and some of these will develop large scale rearing facilities. Botswana, Mali and other countries without their own facilities will purchase flies from one of these countries.

Training and continuing education will be required to develop and maintain technical competence in QC of tsetse. Training includes *in situ* experience, exchanges among cooperating institutions, internships at key locations, fellowships at academic institution etc.

Research support for QC methods should be provided, including expertise at each location. QC personnel help identify and solve local production problems.

Improved communication among facilities producing and using tsetse flies will foster rapid dissemination of quality control and production information, interaction with inter-disciplinary colleagues, rapid feedback from users to producers and easy access to suppliers.

Leaders should be identified and involved in tsetse fly QC, particularly those already experienced in mass production, field evaluation and QC. These leaders will be mentors.

Experience gained in QC for tsetse will easily transfer to other pests programmes and vice versa.

2.3. Current Quality Control Methods for Tsetse Fly

The small scale rearing conducted up to the present (colonies of less than 1,000,000 females , producing about 100,000 sterile males per week) has not required extensive quality control monitoring. Many quality issues were handled informally by the rearing staff, who had a good feel for when the colony was performing well. As colonies expand though no individual will be able to retain an overall view.

Within the colony itself, the parameters being regularly monitored are daily mortality, fecundity and pupal emergence. Changes in daily mortality can indicate problems with the holding conditions, feeding conditions or blood contamination. Fecundity (as pupae per female per ovarian cycle) reflects the nutritional quality of the blood and holding conditions, and when expressed as pupae per initial female indicates the overall performance of the colony.

For sterile male release, the parameters monitored are sterility, mortality, fliers (the number of flies flying from the emergence box within 5 minutes), sexing error and marking efficiency.

2.4. Existing QC Methods for Other Insects Mass-Reared for SIT

Through considerable research and development, quality control procedures for the expanded production of tsetse fly can be adapted from other arthropod species that are mass produced for SIT. Some quality control methods can also be derived from current tsetse fly rearing programs. Other insects from which procedures can be derived include tropical fruit flies, e.g., the Mediterranean fruit fly, Mexican fruit fly, Caribbean fruit fly, and melon fly; certain Lepidoptera, e.g., pink bollworm, and codling moth; the sweet potato weevil and the screwworm fly. In every case, quality control procedures are used to monitor life history and behavioural traits essential for the mass production and field performance of sterile males. Life history traits typically include measurements of fertility, fecundity, rate of development, size and survival. Behaviour, such as ability to fly, find wild females, and compete with wild males, is more difficult to evaluate. Nevertheless, tests to monitor these kinds of behaviour

must be developed and conducted to assure that tsetse fly males are functional after production, transport and release. As with all arthropod species mass reared for SIT, a quality control system for tsetse fly will encompass planning and administration, design and change control, quality of materials, production control, user contact and field performance, feedback and corrective action, and employee selection, training, and motivation.

3. CO-ORDINATED RESEARCH PROJECTS ON TSETSE FLY

3.1. Previous CRPs on tsetse fly

The sub-programme has run seven previous CRPs related to tsetse fly SIT. The first of these on the general development of the SIT (D4.20.01) to tsetse control in the 1970s was followed by two further CRPs on field application (D4.20.03, D4.20.04) in the 1980s and early 1990s. In addition there have been four CRPs on more specific topics, "Using radiation and isotopes to develop diets for mass rearing haematophagous insects for sterile insect release and to study disease transmission by these vectors" (D4.20.02, completed 1984), "Automation in tsetse fly mass-rearing for use in the Sterile Insect Technique" (D4.20.06, completed 2001), "Genetic applications to improve the SIT for tsetse control/eradication including population genetics" (D4.20.05, completed 2002) and "Improved attractants for enhancing the efficiency of tsetse fly suppression operations and barrier systems used in tsetse control/eradication campaigns" (D4.20.08, ongoing). Both the Genetic application and Automation CRPs have relevance to quality control issues, but neither has addressed quality control issues directly.

3.2. Recommendation for a new CRP

A Consultants Group Meeting, comprising a multi-disciplinary team of QC experts, convened in Vienna 10 – 14 June 2002 to assess research and development needs in the field of QC for tsetse SIT. They examined the current status of knowledge and various QC options for use in producing and releasing tsetse fly and other arthropods.

Their conclusions were:

4. Unlike the previous CRPs, which addressed the general application of SIT for tsetse control, odour attractants and genetics, a new CRP should focus on the improvement and harmonization of current QC methodology and the development of QC methods suitable for rapid expansion and long term tsetse production capability and field assessment.

5. A Co-ordinated Research Project focused specifically on QC to address these issues is worthwhile and justified. Achieving the objectives of the CRP is essential for the expansion of tsetse SIT.

3.3. Beneficiaries

The initial beneficiaries of the CRP will be expanded and improved tsetse SIT projects, that are more effective in reaching their goal of establishing tsetse-free areas in mainland Africa.. The ultimate beneficiaries will be the rural population of tsetse-affected sub-Saharan African Member States.

4. NUCLEAR COMPONENT

Sterilization is accomplished by exposing insects to a specific dose of gamma radiation emitted by radioisotopes (Cobalt 60 or Caesium 137). No other methods are available or

appropriate to achieve sterilization. Chemosterilants carry a high risk for environmental contamination and pose unacceptable health risks. Linear accelerators have not shown sufficient applicability and reliability in consistently achieving the desired level of sterility.

Nuclear technology has not only a comparative advantage in sterilizing mass reared insects, but is, at present, the only technology available for this purpose. As every single insect used in SIT activities must be sterilized, irradiation is a central and indispensable part of the total process. Irradiation is also used to decontaminate blood for tsetse diet.

5. OVERALL OBJECTIVE OF THE CRP

Improve and harmonize QC for expanded tsetse production, sterilization and field application.

6. SPECIFIC RESEARCH OBJECTIVE (PURPOSE)

- To improve existing quality control procedures and methods.
- To develop new tests and standards, particularly in the areas of reproductive behaviour, mating compatibility, field performance, irradiation and dosimetry.
- To harmonize quality control procedures among tsetse production facilities and areawide SIT programmes.

7. EXPECTED RESEARCH OUTPUTS (RESULTS)

(Outputs are not ranked in any order of importance)

1. Agreed standardized quality control protocols for reproductive behaviour, including field cage and other tests of sound production and detection, courtship, mate location, strain compatibility, strain competitiveness, re-mating, odour cues/attractants, and mate selection.

2. Agreed standardized operating procedures and associated quality control protocols for tsetse fly blood diet and feeding, including collection, decontamination, nutritional, bacterial and drug residue analysis, disease risk amelioration (BSE etc.), storage handling and feeding. Standards for additives to fresh blood and for synthetic and semi-synthetic diets should also be developed.

3. Agreed standardized operating procedures and associated quality control protocols for irradiation, including stage of development when irradiated, irradiation conditions (temperature, atmosphere), dose, dose uniformity and dosimetry.

4. Agreed standardized operating procedures and associated quality control protocols for fly release, including handling, sexing accuracy, marking, packaging, transport and dispersion.

5. Agreed standardized quality control protocols for released flies, including flight ability, flight muscle development, nutritional status, vector competence and age at release.

6. Agreed standardized operating procedures and associated quality control protocols for colony maintenance, including strain management and compatibility, strain

establishment, disease control (e.g. salivary gland hyperplasia virus), sex separation, feeding, and performance monitoring

7. Agreed standardized system for quality control, including facilities, equipment, staff, training, reporting procedures and responsibilities.

8. Harmonization of existing quality control procedures.

9. Publication of results and securing of intellectual property arising from the project.

8. ACTION PLAN (ACTIVITIES)

Activity 1. Form network of researchers to address the issues identified above.

Activity 2. Award Research Agreements and Technical and Research Contracts.

Activity 3. Organise 1st RCM to refine the logical framework and co-ordinate research areas and methods.

Activity 4. Organise 2nd RCM to review results and refine approaches.

Activity 5. Organise 3rd RCM to review results and refine approaches.

Activity 6. Organise 4th and final RCM to assess the success of the CRP, collate all reports and synthesise results.

Activity 7. Publish the results of the CRP.

Researchers from all tsetse-infested countries and other research institutes working on topics relating to tsetse quality control, such as behaviour, procedures, equipment and diet, should be included and are possible candidates for the CRP.

9. INPUTS

6 Research Contracts	US$ 6,000/year for 5 years	US$180,000
2 Technical Contracts	US$15,000/year for 5 years	US$150,000
4 Research Agreements		
4 Research Co-ordination Meetings	US$30,000/meeting	US$120,000
		US$450,000

10. ASSUMPTIONS

- Continued interest of FAO and IAEA Member States in the development of environment friendly alternatives to tsetse control.

- As a result of the PATTEC initiative and FAO and IAEA resolutions in support of PATTEC, tsetse fly production and the application of SIT against tsetse will be expanded significantly.

11. FORMAT FOR THE LOGICAL FRAMEWORK

(Project No. E4.02, Activity 1)

Narrative Summary	Objective Verifiable Indicators	Means of Verification	Important Assumptions
Overall Objective: To improve and harmonize QC for expanded tsetse production, sterilization and field application.	Expanded application of areawide SIT against tsetse	Country reports	Continued interest of Member States in the development of environment friendly alternatives to tsetse control Strong commitment of African member sates to PATTEC
Specific Objectives: 1. To improve existing quality control procedures 2. To develop new tests and standard, particularly in the areas of reproductive behaviour, mating compatibility, field performance, irradiation and dosimetry 3. To harmonize quality control procedures among tsetse production facilities and tsetse areawide SIT programmes	1. Improved QC procedures available in the areas of viability, fecundity, pupal eclosion and induced sterility 2. New tests available in the areas of reproductive behaviour, mating compatibility, field performance, irradiation and dosimetry 3. QC procedures harmonized and in use by tsetse production facilities and tsetse areawide SIT programmes	1. QC Manual available with improved procedures in the areas of viability, fecundity, pupal eclosion and induced sterility 2. QC Manual including new tests in the areas of reproductive behaviour, mating compatibility, field performance, irradiation and dosimetry 3. QC Manual reflecting harmonized/standardized quality control procedures	Tsetse fly facilities and production will be expanded significantly Member States willing to use improved and new quality control tests and to harmonize quality control procedures
Outputs: 1. Quality control protocols for reproductive behaviour	Quality control standard developed	Standard published	Development of standards is feasible Colony and wild flies available
2. Quality control protocols for tsetse fly diet	Quality control standard developed	Standard published	Irradiation facilities available Blood is available to the researcher

Narrative Summary	Objective Verifiable Indicators	Means of Verification	Important Assumptions
3. Quality control protocols for irradiation of tsetse flies	Quality control standard developed	Standard published	Irradiation facilities available
4. Operating procedures for fly handling, transporting and release	Standard operating procedure developed	Standard published	Field cages available
5. Field quality control protocols for released flies	Quality control standard developed	Standard published	Release equipment available
6. Quality control protocols for colony maintenance	Quality control standard developed	Standard published	Access to colonies possible
7. Standardized facilities, equipment, and materials for quality control	Standard developed	Standard published	
8. Harmonization of quality control methods	Quality control manual developed	Manual published.	Harmonization of quality control methods is agreed
9. Publication of results	Publications		
Activities:			
1. Form network of research collaborators interested in tsetse QC	Research contracts and agreements awarded.	Approval of contracts and agreements by PCC-NA sub-committee	Suitable proposals submitted and funds available.
2. Organize the 1st RCM to refine the logical framework and co-ordinate research areas and methods	1st RCM held 2003	Participants and revised logical framework	Collaborators have access to tsetse fly colonies.
3. Organize the 2nd RCM to review progress and refine approaches	2nd RCM held 2005	Progress reports	Progress is satisfactory
3. Organize the 3rd RCM to review progress and refine approaches	3rd RCM held 2006	Progress reports	Progress is satisfactory
4. Organize the Final RCM to collate all reports and synthesise results.	4th RCM held 2008	Final report	Final reports are submitted by participants to Agency.
5. Publish the results of the CRP		Publication	

12. BRIEF SUMMARY FOR THE AGENCY'S BULLETIN

The recent Pan-African Tsetse and Trypanosomosis Eradication Campaign (PATTEC) provides a mechanism within which SIT will be one of the major components of an integrated areawide approach to the establishment of tsetse fly-free areas. Currently world-wide tsetse production is 1/40 of the projected requirement in 2006. To achieve this objective it is essential that quality control (QC) measures suitable for the expanded production be in place. Therefore, improved QC methodology has become a top priority. Improvements in QC methodology will help to ensure the attainment of these production goals and improve quality of rearing, minimize production costs and generate trained QC and production staff required to successfully produce flies and monitor their quality and suitability for release. The proposed CRP is designed to address these issues.