Title of the Co-ordinated Research Project:

Improved and Harmonized Quality Control for Expanded Tsetse Production, Sterilization and Field Application

Section/Division: NAFA

Project Officer: Feldmann Udo/Parker Andrew

Period Covered: 2003-06-12 through 2009-09-30

Objectives of CRP:

(a) Overall (Agency Project towards which CRP directed):
Improve and harmonize QC for expanded tsetse production, sterilization and field application.

(b) Specific (CRP):

- 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.

Outputs:

(a) Research:

Quality control protocols for reproductive behaviour

- Based on the studies of reproductive behaviour a quality control protocol has been drafted on mating time.
- Protocols for field cage mating and compatibility tests were produced. Further work is required on how to observe and record mating behaviour.

Quality control protocols for tsetse fly diet

- A variety of collection procedures were tested either with the use of defibrination or anticoagulants.
- A draft protocol has been developed for the use of feeding/taste stimuli for feeding of wild collected flies.
- The protocol for blood collection will be updated to include blood collection with anticoagulants.
- A draft protocol for blood storage has been developed.
- The protocols for microbial screening of blood, thawing and blood portioning, will be updated.
- A protocol on pasteurization equipment will be drafted, subject to the completion of the relevant contract.

Quality control protocols for irradiation of tsetse flies

- Protocols for field cage tests, suitable for assessing the competitiveness of irradiated males, have been developed.

Operating procedures for fly handling, transporting and release

- A protocol to harmonize isometamidium treatment before release was proposed. A protocol to assess the efficacy of other potential prophylactic trypanocidal drugs was proposed.

Quality control protocols for colony maintenance

- A QC protocol was drafted to analyse song activity in tsetse. The basic research was completed but it has not been demonstrated if the effect is generic or species specific. Additional data is needed to support the protocol, and appropriate standard developed, perhaps for each species separately.
- A QC protocol was drafted on thorax muscle development.
- A QC protocol was drafted on flight activity using a flight mill.
- Existing rearing protocols for the conventional system and TPU3 were tested in five centres.
- The CRP facilitated the successful adoption of the TPU3 at Kaliti and Bobo Dioulaso
  - for G. fuscipes fuscipes at Kaliti
  - for G. palpalis gambiensis at Bobo Dioulaso
- Use of the TPU3 system reduces labour for feeding by 80% and the personnel needed for growth of colony size.

Standardized facilities, equipment, and materials for quality control

- Pasteurization, flight mill, acoustics recording and software, video recording of mating, wind tunnel, feeding in field cage.

Harmonization of quality control methods

- The FAO/IAEA Standard Operating Procedures for Mass-Rearing Tsetse Flies were circulated to all participants directly involved with fly rearing.
- There was a clear benefit to having simple equipment and simple protocols

(b) Others

Behaviour and development group additional research outputs:
Investigation of the synchrony and circadian periodicity of parturition (larviposition) under different breeding conditions revealed that the larviposition gate (daily peak) differs in three different species investigated (G. pallidipes, G. m. morsitans, G. f. fusiceps). Colonies kept at low light intensity (0.5 – 5 Lux) showed poor daily synchrony of larviposition. Increasing the light intensity ten-fold restored a normal cycle. A simple way to check for normal larviposition time (during photophase) is to compare pupa production during the dark and light phases of the day.

Behaviour of aborted larvae: The behaviour and survival of larvae aborted at various times before the parturition gate was studied. Only larvae aborted after they stopped feeding from the “milk gland” formed normal puparia, but with a considerable delay. Their intrapuparial development was successfully completed, but in several cases adult emergence was postponed by several days in comparison with normally deposited individuals. This interesting phenomenon deserves further investigation, because it may have consequences for timing of emergence in the automated sexing system.

The expulsion of the larva is stimulated by a parturition hormone (PH) present within the female’s uterus. We attempted to reveal the chemical nature of this putative peptidic hormone. Using HPLC we purified the extract of the uterus and by means of MS/MS analysis identified possible amino acid sequences of the most biologically active fraction of the last purification step. The deduced sequences were too short to obtain significant hits both in protein and nucleotide databases. Moreover, the Glossina genome and EST projects are not yet finalized which hampered us to make relevant conclusion. The results will be published, if synthetic peptides based on the identified sequence show PH activity. The fact that extracts of the genital ducts of insects from diverse taxa (the silkmoth Bombyx mori, the migratory locust Schistocerca gregaria, the flesh fly Sarcophaga bullata) also exhibit PH activity in the tsetse bioassay suggests that this hormone or structural homologues may be present in insects in general and play a regulatory role during oviposition/ larviposition. If so, it could be a potential tool for new insect control strategies.

Haemocoelic pulsations before and after parturition: The neuromuscular mechanisms involved in tsetse parturition were studied using a technique of monitoring haemocoelic pressure changes. This provided an overall picture of events accompanying parturition. Muscular activity of the pregnant female intensifies several hours before parturition and ceases immediately after the birth of the larva. Another vigorous muscular activity was regularly recorded some 2 hours after parturition. We assume that this activity is associated with ovulation. Barographic records thus confirmed that parturition is the culmination of a long period of covert preparatory muscular activity in the stationary fly rather than the consequence of a few seconds of muscular labour.

Effects of a dipteran oostatic hormone on reproduction in the tsetse fly: Oostatic or antigonadotropic peptides have been described in a number of insect species. Since they were originally discovered and intensely studied in blood feeding insects – mosquitoes, and later they appeared to be active also in the flesh fly, a representative of the cyclorrhaphous Diptera, we were interested if they can also affect oogenesis and associated gonadal functions in the tsetse female. No apparent differences in feeding behaviour were observed, but larviposition was significantly suppressed in the hormone injected females. Decrease in larval production in injected females was particularly evident during the later gonotrophic stages. This project continues.
Copulatory behaviour in tsetse flies: The description of events occurring during copulation is important for understanding reproduction in tsetse flies. The time spent in copulation prior to accessory secretions or sperm transfer is an important component of the mating experience of female tsetse flies. Experimental alteration of the form of species-specific male genital structures that remain outside the female during copulation affected three potential cryptic female choice responses in Glossina pallidipes and G. morsitans centralis, i.e. sperm storage, ovulation, and female resistance to re-mating decreased. The possibility that these effects were due to changes in male behaviour rather than changes in the stimulation received by the female from male altered genitalia was ruled out, as similar effects were obtained when the female sensory abilities at the sites that are in contact with male structures during copulation were extinguished or severely altered. It is concluded that male mechanical stimulation during mating is likely to be responsible for the female responses. Deductions from previous studies of behaviour, morphology, and physiology that suggested that male genital structures function to stimulate the females to gain paternity were thus confirmed. This is the most complete direct confirmation that sexual selection by cryptic female choice is responsible for the rapid divergence evolution that is typical of male genitalia in Glossina. The results are to be published.

Neuromuscular activity in the female during copulation. Using barographic techniques we monitored fluctuations in haemocoelic pressure, which reflect muscular contractions of the female in copula, and obtained unique records of the female’s participation in mating behaviour. The records will be compared with video recordings of male copulation made by other members of the CRP.

Within the research of the sound production, 3 acoustic signals were tested: feeding sounds, mating sounds and oviposition sounds and several physical parameters were evaluated (frequency, sound pressure level, frequency change, intensity change, time). Song activity was selected as the only useful parameter for quality tests.

Basic metabolic rate (oxygen consumption) was tested as a possible quality criterion. The method has several advantages, i.e. it is an easy method, pupae can be also used and random sample size is possible. For the tests the simple inexpensive Scholander respirometer was used.

Research on the development of flight muscles clearly showed age related changes.

An unusual composition of the direct flight muscles in males of G. pallidipes reared in the facility was observed, i.e. Z-lines were distinctly uneven, M-lines were missing.

An assessment of the effect of in vitro feeding of tsetse flies on the development of trypanosome infections in tsetse was initiated.

Wind tunnel tests: Studies were started on the responses of tsetse to visual and odour cues in a large wind tunnel. The difference between the directed, targeted flight in response to a visual target was contrasted with the untargeted flight response to odour alone.

**Effectiveness of CRP:**

(a) In reaching Specific Objective:
Specific Objective 1. “To improve existing quality control procedures and methods”

Most of the existing protocols were re-evaluated and perfected through the research done, on blood collection, storage and handling and mass rearing. The application of isometamidium chloride to significantly reduced vector competence was investigated but further work is required to confirm the finding.

Specific Objective 2. “To develop new tests and standards, particularly in the areas of reproductive behaviour, mating compatibility, field performance, irradiation and dosimetry.”

Many new or revised protocols were prepared on male quality, acoustic testing, mating time, larviposition circadian pattern and flight muscle development. In addition, known, but infrequently used methods were tested and their usefulness was evaluated. These techniques include flight mill tests, measurements of the residual dry weight and thoracic surface and checks of photoperiodic response in larviposition. Several new QC protocols were drafted.

Specific Objective 3 “To harmonize quality control procedures among tsetse production facilities and areawide SIT programmes”

The FAO/IAEA Standard Operating Procedures for Mass-Rearing Tsetse Flies were distributed to all the participants directly involved in rearing and the procedures were tested and reviewed. Also, through the RCMs two African rearing facilities were visited, so the participants were able to compare the conditions under which flies are reared, and under which the quality tests can be performed. These visits and discussions allowed adaptation of the quality tests for wider use.

The CRP has moved toward standardized blood collection, storage and processing by tsetse rearing facilities in Africa.

(b) In contributing towards Overall (i.e. Agency Project) Objective:

New protocols were developed, some improved, harmonized, adopted., and the need for new standards identified.

(c) Factors, if any, which adversely affected the effectiveness of the CRP:

i) Appropriateness of the CRP

The objectives of CRP were too broad, some participants dropped out, some topics were not addressed since no one volunteered to study those topics.

ii) Formulation of the CRP

Expectations too high given participants available.

iii) Management problems

Late entry of new participants delayed completion of CRP.

iv) Expectations met

Several protocols were developed, new research findings presented, future work identified, resulting in higher quality sterile insect quality. For example, there were inputs into the improvement of many of the blood collection quality procedures.
The CRP gave rise to numerous and appropriate inputs for the mass rearing activities.

**Impact of the CRP:**

- Using QC protocols, mass rearing will now produce more flies of high quality for the SIT program.
- The improvements in the blood collection, processing and storage will lead to the improvement of the tsetse mass rearing for the SIT program.
- Increased communication and cooperation among people involved in research on and production of tsetse flies.
- Interdisciplinary cooperation increased, e.g. engineering, behavioural research.
- New research on relevant behaviour, rearing, etc. elements related to SIT technology.
- Capacity in tropical parts of Africa to rear quality flies increased.
- As a result of highlighting the virus infection in *G. pallidipes*, a new CRP was formed.

**Relevance of the CRP:**

The topic of this CRP was highly relevant to the Agency’s programme to develop the SIT for tsetse control.

Recommended future action by Agency:

1. Further work is needed to refine and improved several of the protocols.

2. Collaborative research in the following subjects should be facilitated:

   a) QC based on acoustic signals: it would be necessary to set control values for flies of good and poor quality for correct evaluation of recorded values. This should be done with all reared species.

   b) Based on the scientific background done developed during the CRP project, it is important to document the duration of mating routinely in mass reared strain as an indirect measurement of the mechanical stimulation that mass reared male are providing to the females. It is also recommended to relate the duration of the mating to the presence or absence of sperm in the spermatheca as well as the percentage of filling. It is also important to document these two parameters during mating compatibility tests.

   c) Concerning the development of the flight muscles, the observations should be made on flies kept in the wild to evaluate the results gained with laboratory flies.

   d) Respirometry was investigated as a prospective method for QC tests. However, only preliminary observations were made; it would be very interesting to continue testing this method for the purposes of the QC procedures.

   e) If production of the colony decreases, environmental parameters of the rearing room including light intensity and duration of light/dark cycles should be checked and circadian periodicity of larval production evaluated.

   f) Tests need to be done to see if pre-mixtures of bovine porcine blood gave the same result as feeding flies bovine and porcine blood on separate days.

   g) More investigation is needed on improving the establishment of wild colonies.
h) More information is needed on competitiveness of sterile males under natural conditions in the different areas where SIT would be applied: (dispersal, mating compatibility, survival, responses to traps).

i) There is a need to assess the vector competence of all tsetse strains mass-reared for SIT.

j) Respirometry: It is possible that measurements of the oxygen consumption and water vapour loss could help with fly handling procedures, i.e. to reduce stress/mortality of flies produced for release and of flies being transported from the field for compatibility studies or for other work in captivity.

k) Larviposition and abortion: Why do abortions occur?

l) Further research on the oostatic hormone in tsetse is required to elucidate its effect and significance in tsetse rearing.

m) Some of the areas to improve blood collection, processing and storage, can still be investigated.

n) Publish protocol on mass-marking of flies for release.

o) Make available the existing protocol on field-cage mating behaviour.

p) A new CRP on sterile male performance in the field should be considered. Include work (e.g. CIRDES) on marking individual insects for mark-recapture studies.

q) Consider developing a single index of sterile male performance.

Publications in press, submitted and in preparation


