Automation for tsetse mass rearing for use in sterile insect technique programmes

Final report of a co-ordinated research project
1995–2001

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FOREWORD

The Insect Pest Control sub-programme of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture has worked for many years on developing the sterile insect technique (SIT) for tsetse fly control. A standard rearing system was developed twenty years ago that allowed the rearing of sufficient flies for research, development and small field operations, such as the BICOT project in Nigeria in the 1980s and the successful eradication of *Glossina austeni* from Unguja Island, Zanzibar in 1997. The research and development work of the Entomology Unit of the FAO/IAEA Agriculture and Biotechnology Laboratory in Seibersdorf, Austria and a number of collaborators through six previous and current co-ordinated research projects has focused on tackling a number of issues critical to furthering the widespread application of SIT. These include using radiation and isotopes to develop diets for mass rearing haematophagous insects for sterile insect release and to study disease transmission by these vectors; improved attractants for enhancing the efficiency of tsetse fly suppression operations and barrier systems used in tsetse control/eradication campaigns; and genetic applications to improve the SIT for tsetse control/eradication including population genetics.

It has become clear though that if the SIT is to be adopted more widely for tsetse control more efficient rearing techniques will be needed. The IAEA and FAO established a co-ordinated research project in 1995 to address this issue and six scientists and engineers from five countries participated. Four research co-ordination meetings were held in which the research programme was established and the results discussed.

This report summarizes the results of the co-ordinated research project. The officers responsible for this publication are E. Opiyo and A. Parker of the Agency's Laboratories, Seibersdorf.

EDITORIAL NOTE

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SUMMARY

The rearing of tsetse flies for the sterile insect technique has been a laborious procedure in the past. The purpose of this co-ordinated research project (CRP) "*Automation for tsetse mass rearing for use in sterile insect technique programmes*" was to develop appropriate semi-automated procedures to simplify the rearing, reduce the cost and standardize the product.

Two main objectives were accomplished. The first was to simplify the handling of adults at emergence. This was achieved by allowing the adults to emerge directly into the production cages. Selection of the appropriate environmental conditions and timing allowed the manipulation of the emergence pattern to achieve the desired ratio of four females to one male with minimal un-emerged females remaining mixed with the male pupae. Tests demonstrated that putting the sexes together at emergence, leaving the males in the production cages, and using a ratio of 4:1 (3:1 for a few species) did not adversely affect pupal production. This has resulted in a standardized system for the self stocking of production cages.

The second was to reduce the labour involved in feeding the flies. Three distinct systems were developed and tested in sequence. The first tsetse production unit (TPU 1) was a fully automated system, but the fly survival and fecundity were unacceptably low. From this a simpler TPU 2 was developed and tested, where 63 large cages were held on a frame that could be moved as a single unit to the feeding location. TPU 2 was tested in various locations, and found to satisfy the basic requirements, and the adoption of Plexiglas® pupal collection slopes resolved much of the problem due to light distribution. However the cage holding frame was heavy and difficult to position on the feeding frame and the movement disturbed the flies.

TPU 2 was superseded by TPU 3, in which the cages remain stationary at all times, and the blood is brought to the flies. The blood feeding system is mounted on rails to make it easier to move, and with a simple locating system it is quick and easy to move the unit to the next set of cages. This system has proved satisfactory for all tsetse species tested, and is being validated for large scale rearing projects in Burkina Faso and Ethiopia.

The use of inserts in cages was also investigated, but with mixed results. In initial tests cardboard inserts increased the usable fly density in cages one and a half to two fold, but cardboard is impractical for routine use and subsequent tests with plastic inserts did not achieve such good results. The use of inserts was not continued, but new developments of cage design may enable inserts to be used in the future.

The overall effect of these changes has been to reduce the total labour required for tsetse mass rearing by more than 50%. Labour has been a major component of the cost of rearing under the traditional system, even in countries with low labour rates. This reduction of more than 50% in labour will result in a 30–70% reduction in overall fly production costs, depending on the local labour costs. Apart from this obvious reduction in cost, the reduced labour also reduces the risk to the rearing from human error.

With a fecundity of 4.03 pupae per initial female (PPIF) for the TPU 3 (compared to 4.37 PPIF for the standard trolley rearing system) TPU 3 will maintain a self-sustaining colony with a good rate of growth and surplus males for irradiation.

Future work needs to focus on a number of factors not yet addressed in this CRP. TPU 3 is the latest refinement to the TPU series but it still uses the standard 20cm diameter cages, and pupal and larval collection is also still carried out manually. Further work is still required to develop a simpler and cheaper cage that does not require the gluing of netting by hand. Efforts should also be made to develop a semi-automatic larval/pupal collection system, noting that larval production continues throughout the 24 hour cycle and the design of the pupal collector should allow pupation of larvae that are produced late during the daily cycle. Facility design and environment control particularly light and heating system for blood also require additional development.

All TPU series 1, 2 and 3 continued to use the standard feeding system, with blood being manually loaded on the feeding trays. A design that would allow for a single loading for feeding of the entire row or for replenishment during the course of feeding without interruption would be ideal. The issue of blood quality, the duration of heating and bacterial contamination would need to be investigated. Use of the TPU 3 has been assumed in designing fly rearing facilities in Ethiopia and Burkina Faso.

Other issues identified by the participants that still need to be addressed include facility design and environment control, particularly light control. However as the scale of fly rearing increases the main issues will shift from the mechanics of production to issues of quality control. A future CRP should focus on these issues.

1. INTRODUCTION

Tsetse flies infest 36 African countries and a total of 9 to 10 million km² in Africa. Throughout this area the disease transmitted by the tsetse fly, trypanosomosis, has a devastating effect on livestock and man.

According to the World Health Organization, over 55 million people living in rural areas of sub-Saharan Africa are at risk of contracting sleeping sickness (human African trypanosomosis). Some 30,000 new cases are reported annually but this does not reflect the real epidemiological situation because of poor surveillance. The estimated number of infected persons is over 300,000 [2]; there are no good estimates of the cost of the human disease in lost productivity, reduced lifespan and medical costs.

Direct losses in meat production and milk yield and the costs of programmes to control trypanosomosis are estimated to be between US $0.6 and 1.2 billion each year [1]. This however represents only a fraction of the total cost of trypanosomosis. The major cost comes from reduced agricultural efficiency due to limited traction power for ploughing and transport, and lost opportunity to integrate livestock with crop production, and to use improved, higher productivity breeds.

All methods available for tsetse and trypanosomosis control or eradication have their limitations. Interventions that were successful in the past such as bush clearing or elimination of wild animals on which tsetse depends for food have been banned for environmental reasons. The indiscriminate use of insecticides for aerial spraying is also restricted. The currently available and environmentally acceptable interventions are:

- Parasite control through the use of trypanocidal drugs and to a limited extent the promotion of trypanotolerant livestock.

- Vector control or eradication using traps and insecticide treated devices and in some cases baited with attractants (devices may also be treated with fungi, insect growth regulators or juvenile hormone analogues), insecticide treated livestock and the sterile insect technique.

Although the strategies for using these options may vary considerably, only a combination of several of the above methods can effectively foster the establishment of viable agricultural systems.

1.1. The sterile insect technique

The sterile insect technique (SIT) involves the sustained, systematic releases of sterile male insects into an indigenous target population over a period of several generations. The mating of sterile males with fertile insects results in sterility in the female throughout her life span. The insects to be released are propagated at special large-scale rearing facilities. Males are sterilized by radiation at the appropriate stage and then taken to the selected area and released. Release is made by air. By continually releasing sterile males in sufficient quantities over time to cover several generations of the target species, its reproductive capacity, and hence the fertile population, is progressively reduced. A point is reached when there are so few fertile insects remaining that fertile matings do not occur and the population is eliminated.

The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture has played a major role in promoting the use of SIT for control of insect pests. Whilst SIT has been very successful for New World Screwworm fly and for the Mediterranean and other fruit flies, tsetse SIT has not been used on a large scale for areawide tsetse and trypanosomosis management. Two early eradication attempts in Nigeria and Burkina Faso initial eliminated the flies, but were not sustainable as the areas had not been selected with due regard to the areawide principle, and support for the programmes was not sustained. Between 1994 and 1996 tsetse were eradicated from Unguja Island, Zanzibar [14], with up to 110,000 sterile males per week being produced by the traditional method. For larger projects however improvements are needed in cost-effectiveness.

The major constraint in these early programmes has been the large scale production of sufficient sterile male flies for release. Tsetse mass rearing is simpler than for most insects as only two developmental stages need to be reared and the only dietary requirement is warm vertebrate blood for adult feeding. However fecundity is very low, and so colony growth is slow and each fly has to be handled individually for sexing, mating and post mating separation. This has made the rearing procedures very labour intensive.

1.2. Tsetse rearing

The design of tsetse rearing system is affected by the fly's reproductive behaviour. Reproduction in tsetse is by adenotrophic viviparity where the female gives birth to a live offspring. The offspring is nourished within the mother by secretions from the highly modified accessory glands and born at an advanced stage of development. The female produces a single offspring at intervals of about 9–10 days depending on temperature, starting

around day 20. The normal maximum adult female lifespan is about 100–120 days, which puts a maximum on the number of larvae one female can produce of about 9. In practice the average achieved in a colony is often around four pupae per initial female loaded into the production cage. This will result in about 1.8 emerged females and the same number of males, assuming a 90% pupal emergence rate, over a period of about 3 months giving at best a 10 fold increase in colony size per year. Pupal production below about 3 per initial female result in no effective colony growth, or even decline, and care must be exercised not to introduce any changes into the rearing procedure that reduce pupal production. Because of the low reproductive rate, large numbers of females are kept for a considerable period of time to produce the excess material for sterilisation and distribution.

Over the last two decades important advances have been made in tsetse rearing. Effective mass rearing procedures have been developed for 7 economically important tsetse species and the membrane feeding system, which eliminates the use of live animals, was developed [3, 4]. This basic tsetse rearing technology using the membrane feeding technique has been transferred to several African insectaries [5, 6, 7].

In spite of these advances, mass rearing remains the most significant area in which improvements are needed before large-scale SIT can be undertaken. In conventional rearing adult flies are held in cages kept on trolleys and cages of flies are manually fed. Sex separation of adult tsetse has hitherto been carried out manually after chilling the flies at 4°C. The process occurs several times during the life of a fly, before maturity and after mating for females and one or two additional times for males [8]. The handling of young flies takes a total of 46% of labour time invested (chilling at adult emergence 23%, mating 6% and chilling for post mating sex separation, 17%). Although labour costs in Africa are not high by international standards, this labour intensive rearing system is not amenable to increasing the production of sterile males to an industrial scale and is subject to production quality assurance issues.

For any improvements to be made towards the implementation of areawide SIT against tsetse flies, improvements in rearing technology are therefore essential. The Joint FAO/IAEA Division sought advice from outside consultants, who identified areas for R&D in tsetse mass rearing. Following this advice the Joint FAO/IAEA Division established a Co-ordinated Research Project (CRP) on '*Automation in tsetse fly mass rearing for use in sterile insect technique programmes*' during the period 1995–2001. The main areas identified for initial

R&D related to increasing the cost-effectiveness of holding and feeding of adult flies and sex separation of adult flies. However, some essential procedures needed to be developed and tested prior to the development of automated rearing systems.

1.3. Objective of the CRP

The objective of the CRP was to improve and upgrade tsetse mass rearing by the development and utilization of automation and other methods.

2. PROCEDURES ESSENTIAL FOR THE DEVELOPMENT OF THE AUTOMATED TSETSE REARING SYSTEM

2.1. Position of cages

Tsetse flies are traditionally held in production cages kept on shelves on trolleys in a horizontal plane. In this plane flies have the option to either perch on the netting on either end of the cage or on the vertical cage wall. During the early part of the development of the tsetse production unit (TPU), the question raised was how the flies would respond to cages held in a vertical position. This prompted investigation during which cages were held with mated female *G. austeni* in a vertical position for 8 weeks and their survival and productivity compared with those of flies kept in a horizontal plane.

Results indicate significantly better performance for both survival and productivity for the flies kept in a horizontal plane. Mean pupae per initial female (PPIF) at 8 weeks was 2.84 and 2.26 and survival was 65.3% and 42% for horizontal and vertical cages, respectively. Flies were observed to accumulate at the bottom of vertically held cages.

2.2. Resident males

In the conventional tsetse rearing, sexes are kept separate from the time of emergence. When mature (females 3–7 days for most species and males 7+ days old), the sexes are placed together in a 1:1 or 1:2 ♂:♀ ratio for 3 days for purposes of mating and then separated again. This procedure involves chill separation of flies at emergence and after mating. As it would also be impractical to handle large colonies following these procedures, a way was investigated of reducing or eliminating the need for separation of sexes after mating by leaving the males in the production cage.

Mature *G. austeni* males were held with females in 1:1, 1:2, 1:4 and 1:8 ♂:♀ sex ratio but keeping the initial number of flies constant at 100 flies per 20 cm diameter cage. Males were left continuously in the production cages for the duration of the experiment and records were kept of the survival of flies and pupal production. A group of 100 female flies that were mated and separated after two days were used as control.

Pupal production in the different groups showed that the lowest production was recorded for 1:1 not separated group with a PPIF of 3.26 at week 11. The highest production was for 1:4 group with a PPIF of 3.75. The control group had a PPIF of 3.5. However, the best female survival was recorded for 1:8 group followed by 1:4 and lowest for 1:1 not separated group. It

is assumed that at the higher ratios of males, the continual aggressive male behaviour resulted in lower female survival and higher abortion rates. At low male ratios though, any detriment from male mating attempts appears to be more than offset by the reduced detrimental effect of chilling as a result of discontinuing the chilling for post mating separation.

2.3. Day 0 mating

Previous laboratory investigations showed that maximum insemination rates are achieved in a number of tsetse species when mating occurs between 3 day old females and adult males more than one week old. By this time the female would have had the opportunity to feed twice and males several times. There are species differences and optimal fertility in *G. pallidipes* is achieved by mating 7–9 days old females with 10 days old or older males, whereas in *G brevipalpis* the females are most receptive on the day of emergence. In the field, however, it is usually very rare to find virgin females in a sample of flies caught in traps or off a bait animal [10]. In addition laboratory reared *G. austeni* have been observed to mate in emergence cages before sexes are separated [11]. Having established that putting 1 male to 4 female *G. austeni* in a production cage does not compromise survival or production, investigations were carried out to determine if it was necessary to mature flies before placing them in cages for mating. Newly emerged *G. austeni* flies at a ratio 1 male to 4 females were put in production cages and their performance compared to that of 2 day old females mated with males which were 7 days old.
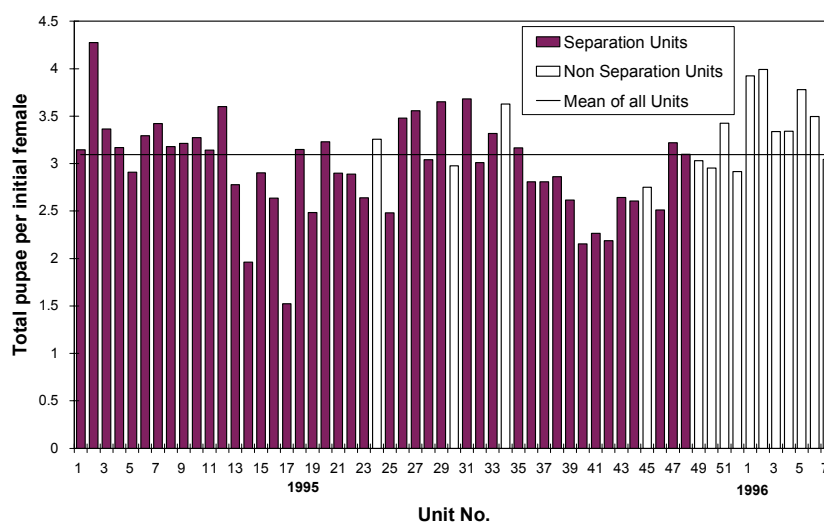

Figure 1. Comparison of day 0 mating separation and non-separation units for G. austeni. *(Separation: Mean = 2.960, sd = 0.507, n = 44; Non-separation mean = 3.323, sd = 0.384, n = 15; for difference of means, t = 2.9024, p>0.01).*

The performance of flies in the two groups was identical (Fig. 1). The results showed that with *G. austeni*, successful mating takes place even when cages are stocked with freshly emerged flies at a 1:4 male to female ratio. Dissections of females from such groups confirmed that by day 10 after emergence, all females were inseminated. Both survival and production were not affected. These steps were incorporated into colony procedures at the Tsetse and Trypanosomiasis Research Institute (TTRI), Tanga for rearing flies used in the Zanzibar project. With the oncoming tsetse eradication project in Ethiopia it was necessary to test if these steps would be suitable for *G. pallidipes*.

2.3.1. Day 0 mating and sex ratio at mating on performance of G. pallidipes

G. pallidipes has been known to have different requirements for successful mating to occur. With this in mind, experiments were designed to determine the effect of Day 0 mating and the optimal sex ratio at mating for *G. pallidipes*.

For this experiment four 20 cm diameter production cages were used per treatment. The cages were set up with flies at the ratios shown in Table I to give a total of 60 flies per cage. The control group consisted of 7 day old females that were mated with 10 day old males for 3 days after which they were chill-separated and placed into production cages. Survival and production of the different treatment groups were monitored for 13 weeks and the results compared.

There was no significant difference in the survival and pupal production when the flies are mated at the time of emergence as compared to those kept until they mature before mating took place.

TABLE I. EFFECT OF DAY 0 MATING AND SEX RATIO AT MATING ON
PERFORMANCE OF *G. pallidipes*

Age at mating (days)		Ratio	Survival (%)	Pupae per initial female	
♂♂	♀♀	♂:♀	Week 13	Week 13	Week 3
0	0	1:4	52.60±13.32	3.31±0.35	0.41±0.41
0	0	1:5	50.50± 3.00	3.54±0.18	0.35±0.06
0	0	1:6	53.92± 9.80	3.08±0.85	0.28±0.11
0	0	1:8	51.89±10.39	2.98±0.83	0.24±0.19
10	8	1:1*	54.59± 6.14	3.47±0.33	0.60±0.08

* pairs were held for 3 days before separation.

In a separate experiment, cohorts of females were mated with males of the same age, 6, 9, 12 days old and then females observed for pupal production for 14 weeks. A control group of newly emerged males and females where males remained in the cage was used as control. Females confined with males at emergence performed better than the other age groups with a PPIF of 4.2. The lowest PPIF of 1.8 was for 6-day old pairs. Male sexual maturity appeared to parallel that of the female [9]. It was observed that the time to first larviposition was the same for all groups, but the group fecundity during the first week (week 3) of production for Day 0 mated females was lower than for the females that were allowed to mature before mating. This was not surprising in that there were fewer males to mate the females and therefore mating was not synchronised. It should be borne in mind that with Day 0 mating and leaving males in female production cages, the second chilling for separation after mating is eliminated. There is therefore no need to handle young males and females in separate small cages prior to mating, steps which took 17% and 6% of labour time invested in rearing respectively for separation and mating. The revised mating procedure was tested with other species and is now the standard mating regime at the Seibersdorf Laboratories.
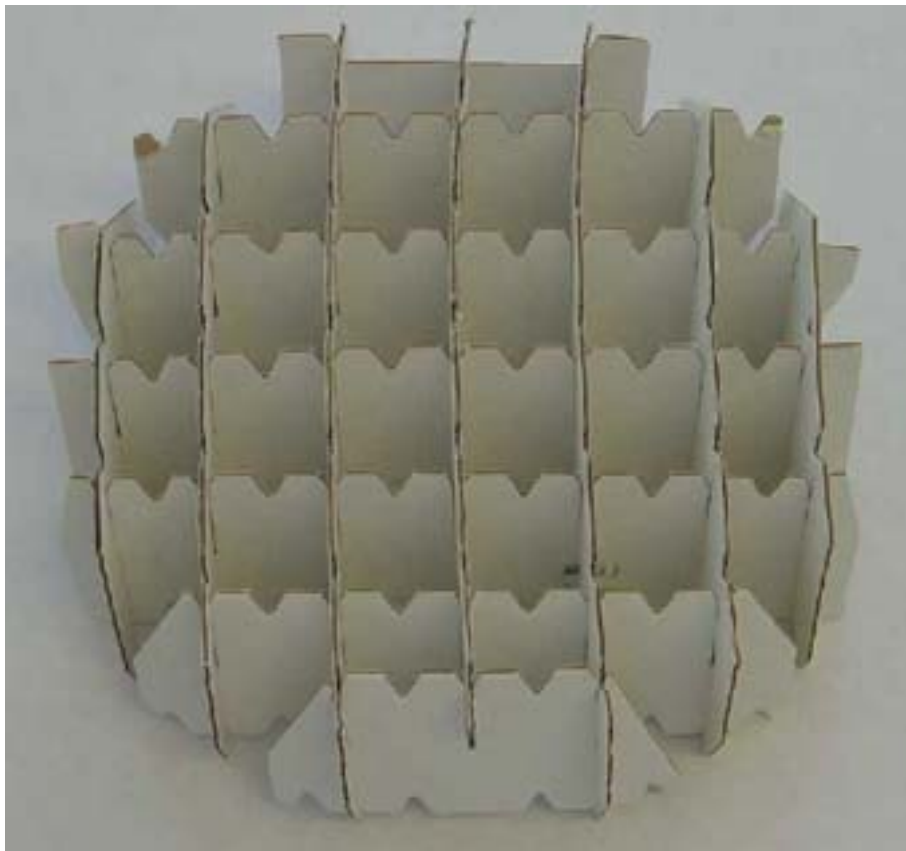

Figure 2. Cardboard insert for standard 200 mm cage.

TABLE II.   RESPONSE OF *G. austeni* TO CAGES WITH CARDBOARD INSERTS

| Cage density | | Total flies/cage | Pupae per initial |
Males	Females		Female (PPIF)
25	100	125	1.79
37	150	187	1.24
50	200	250	1.81
62	250	312	1.72
75	300	375	1.4
87	350	437	0.9

2.4. Inserts

In view of the results obtained with vertical cages where flies were observed to crowd at the base of the cages it was felt that giving flies more resting space by creating cells within the cage would prevent this crowding. This would not only allow for an even distribution of flies within the cage but would also allow an increase in the number of flies that can be kept in the cage. Disposable cardboard inserts (Fig. 2) were used in a preliminary experiment in which cage densities varied from 100 to 350 females per cage at a mating ratio of 1 male to 4 females and leaving the males in the production cages. The results by day 54 are shown in Table II.

Flies were observed to distribute themselves evenly in the cages and there was no harassment of females during feeding. From the results it would suggest that using inserts in cages would double the carrying capacity of cages without compromising performance. However, it should be noted that the inserts made of cardboard are disposable and would have to be replaced from time to time; for this reason in later studies they were replaced by plastic ones, but these did not prove satisfactory and the use of inserts has been discontinued. It is possible that future developments in cage and insert material and design may make the use of inserts once more possible, to increase both fly density and survival.

2.5. Direct loading of production cages

A system is necessary whereby flies emerge directly into a production cage in the right numbers and sex ratio. Two systems were tested based on an inverted funnel fitted with a break-light circuit. This activates a counter when a fly passes through a window to the collection cage above. The system was enhanced by the addition of a computer programme

which shows fly emergence per unit time. This system combined with an imaging process would facilitate experimental research on tsetse emergence behaviour but due to the cost and servicing requirements, does not represent ideal 'transferable' technology and was therefore not pursued further.

The second system required only a top-loading electronic balance and a temperature controlled room and pupae that are collected on a 24 hour basis. The colony cages are weighed and then placed over the required number of mature pupae. As flies emerge into the cage (through the netting) its weight increases. Having obtained the average weight of the newly emerged flies of a given species, the exact number of emerged flies in the cage can be determined. Although this was a simplified approach, the need for a top loading balance to estimate the number of flies for each cage made the approach impractical for large-scale operation. Further development along this line is discussed under self-stocking of production cages (SSPC, see Section 4.4).

2.6. Discussions and conclusions

- The use of inserts in production cages was shown to increase holding capacity for *G. austeni*.

- The mating system using resident males using ratios of 1 male to 4 females was shown not to compromise survival and production. This eliminated one chilling procedure and saves 17% of time invested in tsetse rearing.

- Day 0 mating with resident males using ratios of 1 male to 4 females was shown to be effective. This system removes the need for keeping sexes separate to mature before mating.

- The added advantage of using resident males is that 75% of all males produced are immediately available for sterilizing and release during an eradication project.

- The standard membrane feeding system continued to be used with the rearing systems.

- These procedures were used in the development of the automated rearing system.

3. SYSTEMS FOR HOLDING AND FEEDING LARGE TSETSE COLONIES

The conventional tsetse rearing involves keeping flies in small cages on trolleys. Presently the cages are small, hold a small number of flies (48–80) and have to be manually transferred and placed on membranes for 15 minutes for feeding after which they are returned to the trolley for pupal collection [4]. This limits the number of flies that can be handled at any one time. In
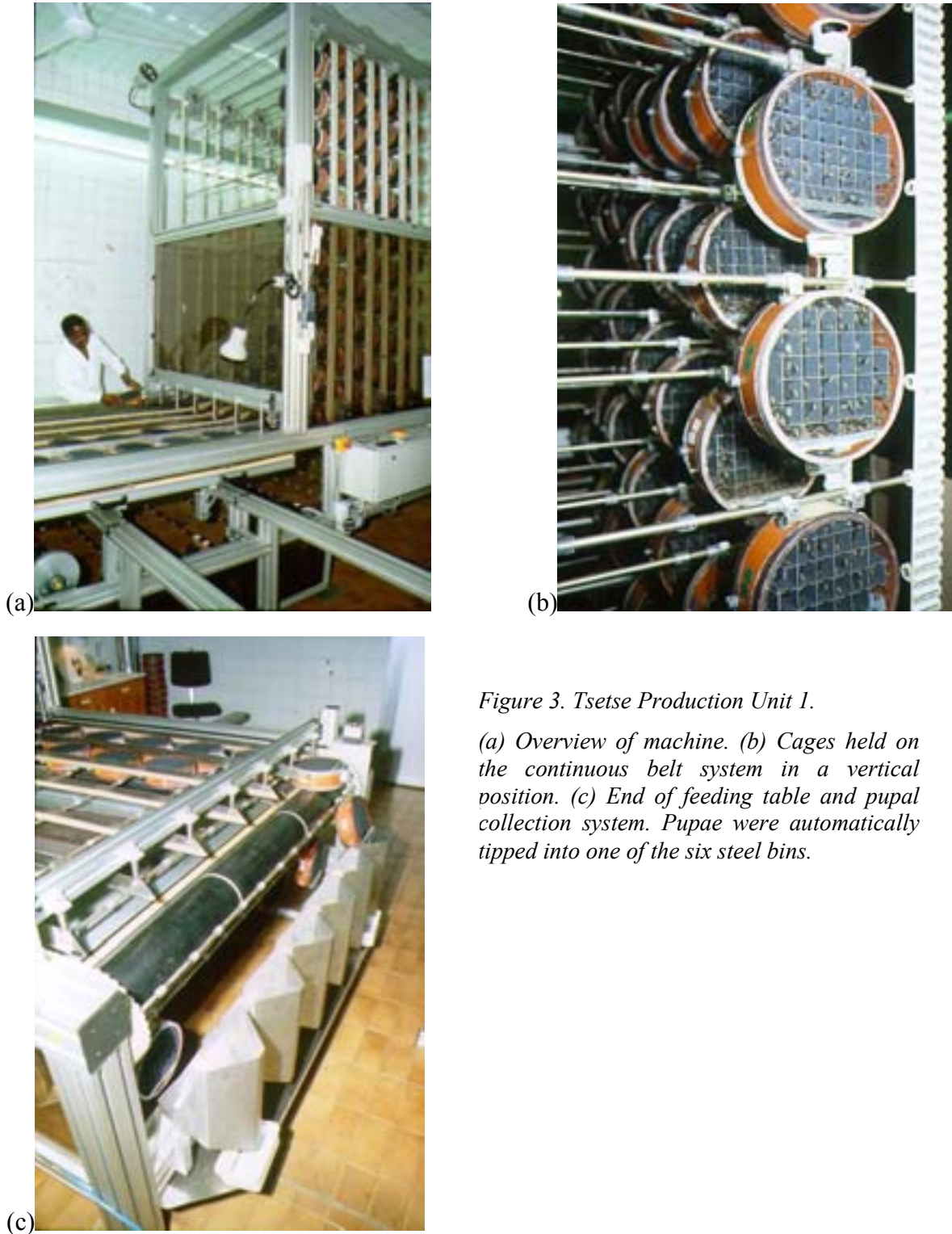
(a)

(b)

(c)

Figure 3. Tsetse Production Unit 1.

(a) Overview of machine. (b) Cages held on the continuous belt system in a vertical position. (c) End of feeding table and pupal collection system. Pupae were automatically tipped into one of the six steel bins.

order to improve these processes in both space and labour needs, a tsetse production unit (TPU) was designed, built and evaluated.

3.1. Tsetse Production Unit 1 (TPU 1)

TPU 1 was a cage transport prototype machine made up of a single unit, occupying some 7 m² of insectary floor space, with storage for 648 production cages and an estimated 225,000 (initial) female fly capacity (Fig. 3). The centralised larval and pupal collection and membrane feeding area are to the front of the TPU 1 and the cage servicing area in the rear. The system could take up to 1,008 cages in a purpose built insectary. Other features of the prototype included independent programming for each of the 6 rows of 108 cages allowing for comparison to be made of diets, feeding frequencies, pupal production and fly density. Up to 6 species of tsetse may be reared simultaneously. The cages were moved constantly through the height and length of the insectary room which together with the use of simple air moving equipment permitted optimal rearing synchronization. Assuming a supply of adequate quality blood, it is estimated that the TPU operation, including cage management and washing up would occupy 35 man-hours a week on a seven-day schedule.

The cage is the central component of any rearing system and its fly holding capacity will determine the efficiency of the total production process. For TPU 1 the 200 mm Ø × 70 mm deep vertically stored PVC cage is closed on both faces by a fine mesh netting to prevent larvae from crawling through (Fig. 4). A plastic cage insert was included which significantly increased the surface area available for fly resting, thereby permitting a greater fly holding capacity. Larval collection was automatic and the operator determined the frequency of collection. A floating membrane system consisting of a pneumatic lift, a full size plywood support covered by Styrofoam insulation, a rubberized temperature controlled heating pad and stainless steel tray held excess blood diet. The predetermined amount of blood, sufficient for the 18 feeding cycles, was poured onto each tray and covered by a membrane that overlapped the sides of the tray. During feeding, 36 cages (six rows of 6 cages each) paused over the membrane covered trays for a pre-set time that commenced when the pneumatic lift raised the feeding table making contact with the cages. After completion of feeding, the feeding table dropped several centimetres while cages with fed flies moved along and the next set of cages could be fed. Feeding was completed in 18 10-minute intervals and thereafter the system would be switched to a slow speed continuous running.

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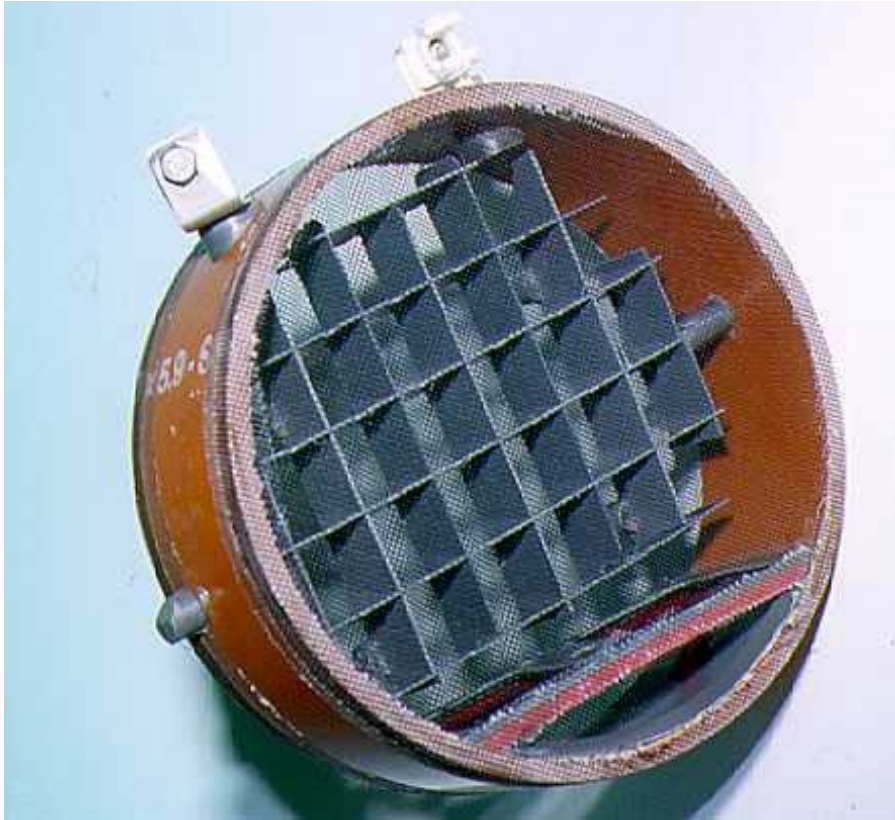
Figure 4. TPU 1 cage, with plastic insert to increase the available resting area.

3.1.1. Evaluation

The evaluation of TPU 1 included cage design, feeding test, fly performance, toxicity and stress tests. Pupal quality was also assessed in terms of size class and weight [6]. *G. austeni* was used to evaluate TPU 1.

a) The feeding efficiency test

The feeding efficiency test was carried out using teneral male flies starved for 48 hours in the standard cage (200 mm diameter × 50 mm deep with 100 female and 25 male flies) and TPU 1 cage (200 mm diameter × 70 mm deep and plastic inserts with 300 female and 75 male flies). Four cages of each type were fed on TPU 1 feeding system and a similar number was fed in the standard colony as control. After 9 minutes of feeding all flies were killed by chilling and examined to determine the number fed. The results showed that flies had access to blood but better feeding was achieved in the standard cage; 91% compared to 87% in TPU 1 cage. In both cases the cages were manually placed on the feeding membrane.

b) Toxicity test

Although flies appeared to have access to blood, the daily mortality rate recorded among flies put on the TPU 1 was 3% (the acceptable daily mortality is <1.2%), and was mainly due to

TABLE III. SURVIVAL OF *G. austeni* IN DIFFERENT TYPES OF CAGES OBSERVED FOR FIVE WEEKS

Cage number	Treatment	Flies/cage	Survival %	PPIF
1	Old TPU, glued&washed, H	300♀+75♂	91.8	0.29
2	Old TPU, glued&washed, V	300♀+75♂	91.2	0.23
3	New TPU, glued&washed, H	300♀+75♂	92.3	0.22
4	New TPU, glued&washed,V	300♀+75♂	76.8	0.17
5	Old TPU, not glued&washed, H	300♀+75♂	89.1	0.35
6	Old TPU, not glued&washed, V	300♀+75♂	88.8	0.17
7	New TPU, no inserts&washed, H	100♀+25♂	93.6	0.38
8	New TPU, no inserts&washed, V	100♀+25♂	88.8	0.48
9	Old STD, no inserts&washed, H	100♀+25♂	92.0	0.58
10	Old STD, no inserts&washed, V	100♀+25♂	89.6	0.54

H = horizontal; V = vertical; TPU = TPU cage; STD = standard cage.

starvation. An investigation was undertaken to see if any component used in cage making was responsible for the heavy mortality. For these tests flies were put in cages with different treatments and observed for 5 weeks. One set of cages was placed in a horizontal (H) position while the second set simulated the vertical (V) plane as on TPU 1 (Table III).

There was no obvious toxic effect from any of the cages indicating that neither the material used for cage housing, inserts in cages or glue were toxic to flies. Flies in cages that were kept horizontally once more performed better than those held vertically.

c) Fly density and movement

After demonstrating that neither the material used in cage making nor lack of access to blood was causing mortality among young flies, it was decided to test the effect of density of flies per cage and movement of these cages on fly performance. On TPU 1 there were basically two types of movement; one 'fast' to bring flies to feed and a second 'slow' to enable cages to experience the same environmental conditions throughout 24 hours.

Six TPU 1 cages with inserts with varying numbers of flies ranging from 100 females and 25 males to 350 females and 87 males on TPU 1 were observed for survival and pupal

TABLE IV. EFFECT OF DENSITY AND MOVEMENT ON PERFORMANCE OF FLIES
IN 10 WEEKS

Cage density	% Mean survival	Pupae per initial female (PPIF)	% Daily mortality Week 1
With movement			
100 f + 25 m	40.60	1.55	2.1
150 f + 38 m	39.77	1.46	2.32
200 f + 50 m	37.38	1.36	2.16
250 f + 63 m	29.02	1.35	3.03
300 f + 75 m	28.29	1.18	2.5
350 f + 88 m	25.89	0.95	3.47
Without movement			
100 f + 25 m	66.30	2.69	1.2
300 f + 75 m	51.08	2.21	1.0

production. Control cages with 100 females and 25 males and 300 females and 75 males were kept in a vertical plane but not on TPU 1 and they were manually fed. Tests were replicated 4 times. In cages held on TPU 1 (continuous movement) the mean % survival decreased with the increase in fly density. The mean daily mortality rate ranged between 2.1% and 3.47% during the first week. Among the flies in cages which were not on TPU 1 and which were manually placed on the feeding membrane, mean survival at the lowest density was 66.3% compared to 40.6% on TPU 1 and the difference increased with increasing density (Table IV).

Pupal production was also better among flies that were fed manually and was lowest for cages with the highest density, PPIF of 0.95. Production in cages which were fed manually (without movement) was 75% more than in TPU 1 cages. The conclusion was that the daily mortality rate did not vary with density of flies on the TPU 1 except at the highest density which had an even higher mortality. The observations lead to the discontinuation of cage movement except during feeding.

d) Insert designs and fly density

In a further search for the likely causes of death among flies maintained on the TPU 1, insert designs were modified in the standard TPU 1 cages and performance of flies monitored for 10 weeks.

TABLE V.   EFFECT OF INSERT DESIGN IN TPU 1 CAGES ON PERFORMANCE OF
            G. austeni

	Density	No insert	Half Insert	Half Division	Normal Insert
% Survival	100 ♀♀	48.68±17.60a	65.00±14.93a	69.0±13.48a	51.60±9.75a
	300 ♀♀	20.71±11.74a	43.14±12.01b	48.75±13.85c	37.50±14.18d
Fecundity	100 ♀♀	0.24±0.13a	0.28±0.10a	0.28±0.12a	0.22±0.10a
(P/F/10d)	300 ♀♀	0.16±0.27a	0.15±0.08a	0.16±0.06a	0.13±0.06a

Means in one row followed by the same letter do not differ significantly, Tukey's HSD test, p.=.0.05.

The modifications of inserts were as follows:

- Normal insert   cells 25 mm × 25 mm for the full depth of the cage
- Half insert   cells as for normal insert but only half the depth of the cage
- Half division insert   cells 55 mm × 55 mm for the full depth of the cage.

Cages without inserts were also included as a control. Two densities of flies per cage were used; 100 females with 25 males and 300 females with 75 males. Mortality and pupal production on the TPU 1 were recorded weekly and tests replicated four times. Data was analysed using Tukey's HSD tests (Table V).

There was significantly better survival of flies in cages with 100 females compared to 300 females. At 100 females per cage, there was no significant difference in survival between the groups although survival appeared best in cages with half division. In cages with 300 females the differences between the survival means were significant (p<0.05) for all treatments. Fecundity was generally poor for all treatment groups at the two densities but cages with the lower density still performed significantly better. Casual observations revealed large number of expelled eggs and aborted larvae in the pupal/larval collector of TPU 1.

3.1.2. Large scale evaluation

Large scale evaluation of TPU 1 was carried out using *G. austeni* during which cages were loaded with freshly emerged flies from the main colony, 300 females with 75 males per cage and fed automatically. At intervals dead flies were removed from the cages and counted. Pupae were collected daily except Sundays and counted. A sample of the pupae was sorted by size and weighed individually in each size class. The same batch of pupae was allowed to emerge in order to estimate the eclosion rate and the sex ratio.

The performance of flies was followed by generation. Because of specific limitations in the cage design it was not possible to accurately determine the adult mortality rate by simply removing flies as is done in standard cages and therefore some assumptions were made in calculating these numbers. As the pupal production index is also related to fly survival this figure also contains an error factor. An additional change was 5 day feeding per week with extended feeding time on Monday and Friday. A large number of flies was introduced on the TPU 1 but it was not possible to establish a self-sustaining colony. This was due almost entirely to the high daily mortality (mean 2.89%). The mean daily mortality was 0.76% for a similar period among flies maintained in the routine colony. In a period of 12 months (July 1996 to June 1998), 180,743 flies were added to the TPU 1 with only 166,928 pupae being produced. The fecundity of the flies that survived was 0.67 pupae per female per 10 days (P/F/10d) for TPU 1 flies compared to 0.86 P/F/10d for the colony flies, slightly lower but not significantly different. This again indicates that female survival and not productivity was the key element, in other words the flies that survived the initial period on the machine were quite productive.

TABLE VI. DISTRIBUTION (%) OF SIZE CLASS OF *G. austeni* PUPAE FROM THE STANDARD COLONY AND TPU 1 G_0 AND F_1 FLIES.

	COLONY	TPU 1 G_0	TPU 1 F_1
Class A	02.08±1.42	10.56±1.62	13.67±1.7
Class B	24.01±1.37	44.94±1.64	46.28±2.08
Class C	62.85±1.50	40.94±1.92	36.31±2.18
Class D	10.99±1.35	03.32±2.04	03.65±2.15
Class E	01.03±1.60	00.24±1.48	00.09±2.41

TABLE VII. MEAN WEIGHT (mg) OF *G. austeni* PUPAE FROM COLONY AND TPU 1 G_0 AND F_1 FLIES

	COLONY	TPU 1 G_0	TPU 1 F_1
Class A	12.93	12.28	12.31
Class B	16.94	16.18	15.86
Class C	20.45	19.46	19.27
Class D	23.49	22.62	22.34
Class E	26.64	24.95	22.90

TABLE VIII. SIZE CLASSES OF *G. austeni* PUPAE (%) BY GENERATION ON TPU 1
WITHOUT CONTINUOUS MOVEMENT

	G0	F1	F2	F3	F4	F5	COLONY
Class A	3.2	7.4	8.6	7.7	3.3	2.8	2.1
Class B	29.9	23.5	31.4	27.5	16.7	29.2	24
Class C	53.5	60.3	54.3	51.6	62.6	45.8	62.8
Class D	11.8	7.4	5.7	14.1	13.2	16.7	11
Class E	1.6	1.5	0	0	2.2	5.6	1

The class sizes and weights and emergence rate of pupae produced by flies on TPU 1 were compared with those from the standard colony flies (Table VI).

The A class pupae which are the smallest pupae made up 10.56±1.62% and 13.67±1.7% of TPU 1 G_0 and TPU 1 F_1 total pupae, respectively, compared to 2.08±1.42% from colony pupae. The mean weight of pupae produced by flies on TPU 1 was generally lower than that from the colony and deteriorated with subsequent generations (Table VII).

The sex ratio at emergence was the same in all the three groups being 1:1, but the emergence rate was 90.39% for colony pupae, 78.54%, TPU 1 G0 and 78.96%, TPU 1 F1. There were also more crippled flies at the time of emergence from TPU 1 produced pupae.

The indications were that the poor eclosion rate and high percentage of crippled flies could have been due to high mortality of pupae in the early stage during development. Dissections of pupae from which flies failed to emerge confirmed that death occurred early during development. The quality of pupae produced on the TPU 1 was certainly inferior and this could have been due to the physiological status of the mother (poor feeding or stress from movement of cages) or the handling/movement during the sensitive stage of melanization and hardening.

A decision was made to abandon the continuous movement. As a result pupae and larvae remained motionless in the cage until just before feeding (when the cage delivers them to the central collector). There was an indication that the pupal quality improved with less pupae being in class A (Table VIII).

3.1.3. Feeding tests

The standard *in vitro* feeding of tsetse flies is through the silicone membrane which is placed on a tray of blood warmed to mammalian body temperature. For TPU 1 the feeding system initially involved moving blood through a closed system either by gravity or peristaltic pump. Blood was warmed as it passed over heated surface which was covered by silicone membrane. Two problems were identified with this feeding system; although flies actively probed through the membrane, the negative pressure on the blood did not allow flies to engorge and it was difficult to attain the required level of sterility of the feeding equipment. This led to the adoption of a floating membrane for the TPU 1 feeding system.

3.1.4. Discussion and conclusions

The overall productivity of TPU 1 was unacceptable as more flies were going onto the TPU 1 than pupae were being produced. It was clear that without major changes in cage construction and fly holding the TPU 1 would not be developed into a practical unit for rearing tsetse. The inability to collect accurate data on important biological parameters was another major design fault in the machine. However, several related refinements have resulted in significant improvement in tsetse rearing so that production can be increased without a concomitant increase in labour or facilities.

Despite the shortcomings, the TPU 1 did demonstrate that the principles of automated feeding and pupal collection are indeed valid. Experience gained from TPU 1 led to the development and design of TPU 2.

3.2. Tsetse Production Unit 2 (TPU 2)

The first prototype tsetse production unit (TPU 1), a system that automatically handled cages of flies and brought them for feeding while pupae/larvae were collected centrally was found to be inadequate for maintaining a self-sustaining colony. However, the system demonstrated a possibility of automatic handling and feeding of a large number of flies. These principles were further developed into TPU 2.

TPU 2 comprises of a cage holding frame, a pupal collector and a feeding station (Fig. 5). The cage holding frame for 63 cages in 7 shelves measures 228 cm long, 69 cm wide and 233 cm high. At the time of feeding, the frame is moved to the feeding table to which it interlocks, the cages are then simultaneously lowered onto the feeding trays by manually moving a lever

Figure 5. Tsetse Production Unit 2. (a) Cage holding frame, with 7 levels of 9 cages each. (b) Pupal collection unit. (c) Rear of cage holding frame to show the lever system to lower the cages onto the feeding membranes.

system and after 15 minutes the cages are raised and the frame is moved back to its original position (Fig. 6).

Rectangular cages measuring 20 cm × 60 cm × 7 cm with fine netting on the top and a coarse netting at the bottom are held horizontally on clamps. Movement of flies/cages is limited to the time of feeding only. Shelves on the pupal collector provide a surface onto which larvae drop from the cages and then roll into a trough where they pupate. The angle of the shelf is adjustable to allow for the removal of the pupal collector during the time of feeding (Fig. 7).

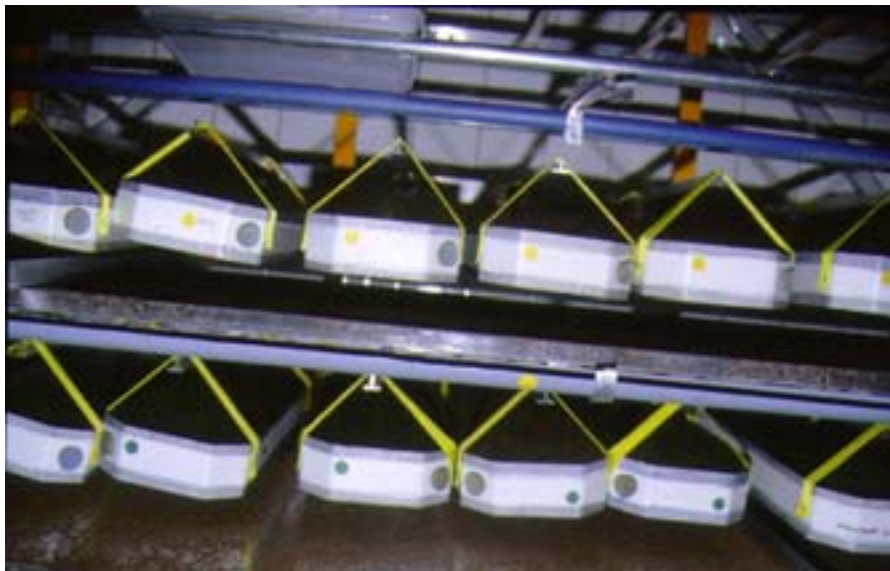*Figure 6. TPU 2 cages being inserted into the feeding station.*

Figure 7. Pupal collection system under TPU 2 cages.

3.2.1. Evaluation

The basic parameters used for evaluating performance of flies on TPU 2 were the same as for TPU 1.

a) Cage density

Several fly densities were tested in the new cages to establish production criteria. Densities ranged from 260 females to 600 females at a sex ratio of 1 male to 4 females. Some cages had more resting surfaces created by use of benches (three horizontal plastic bars about 50mm wide) inside the cages. A density of 260 females and 65 males in the rectangular cages is equivalent to the density/volume in the standard 20 cm diameter cage without inserts that holds 48 females and 12 males.

TABLE IX. PERFORMANCE OF *G. pallidipes* IN TPU 2 CAGES AT DIFFERENT
DENSITIES AFTER 10 WEEKS

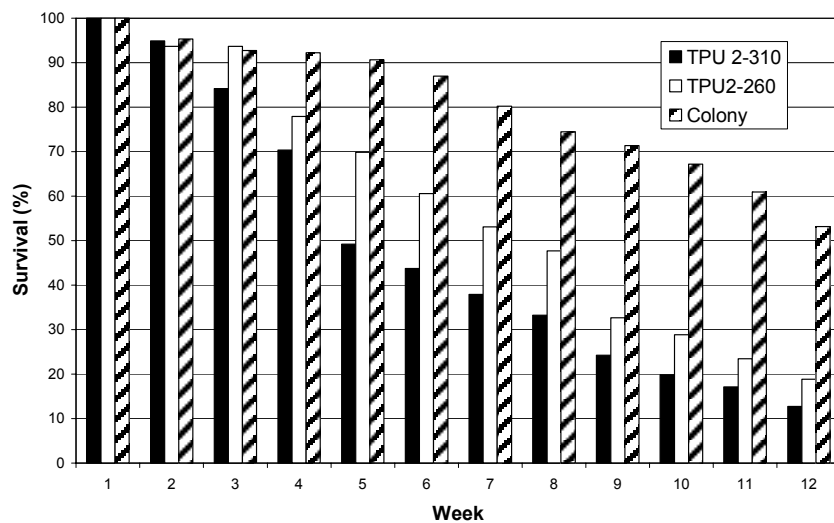| Density of females/cage	% Survival	PPIF
600	11.00	0.39
450 with inserts in cage	4.22	0.67
450	10.67	0.53
360	16.39	1.10
260	29.81	1.96
48 in standard round cage	64.58	2.19

Figure 8. Survival of G. pallidipes *flies at two densities in TPU 2 cages compared to control (200 mm cages on trolleys).*

A 10 week comparison of the rectangular cage with the standard round cage with 48 females and 12 males showed that fly survival was dependent on density. At 260 females/cage only 30% of flies were alive after 10 weeks while in the standard cage it was 65% (Table IX).

Following this observation a set of 9 rectangular cages was loaded with either 260 females and 65 males or 310 females and 78 males per cage and monitored on the TPU 2 for survival and productivity during a period of 13 weeks. Flies were fed 5 days a week and mortality was monitored using two cages from each group on a weekly basis starting at week 2. Quality control was run on pupae which were produced by flies kept on TPU 2. Pupae collected on a daily basis were kept in the different treatment groups and sorted according to size classes 3 days a week. Pupal weights in mg were taken 24 hours after collection. Pupae from the main colony were used as control.

i) Survival

Cages with 260 females and 65 males demonstrated higher survival compared with those of 310 females and 78 males. However, the survival was still below that of the standard colony where the daily mortality was 0.54% compared with 0.89% for cages with 260 females and 0.95% for 310 females. A closer look at the mortality figures showed comparable survival till week 4 after which there was a faster decline in rectangular cages. This coincides with the period when females drop the first larva (Fig. 8). It was also noticed that the distribution of flies in the cages was not uniform with significant crowding taking place that was probably influenced by light direction.

ii) Productivity

Productivity was measured as PPIF. Cages with 260 females had a PPIF of 2.24 compared to 1.18 for cages with 310 females and 3.5 for the standard cage.

iii) Pupal quality

From the results it would appear that the pupae produced by these flies were of comparable quality with those of the colony flies. This was true of the proportion in each class size and also of the mean weights for each class (Table X).

Pupae from TPU 2 flies were set aside to monitor the emergence rate and sex ratio at emergence. A sample of pupae from the colony was used as control. The results indicate that

TABLE X. PUPAL WEIGHTS (mg) OF *G. pallidipes* MAINTAINED ON TPU 2 AND COLONY FLIES

| | Class Sizes | | | | |
	A	B	C	D	E
Colony	22.07±2.66	29.62±2.27	33.03±2.37	38.59±2.05	41.70±2.72
TPU 2-260	21.56±2.16	29.51±1.99	33.91±2.16	38.33±2.03	41.41±2.54
TPU 2-310	23.83±0.31	29.69±1.66	34.75±2.30	38.40±2.78	40.31±2.81

TABLE XI. EMERGENCE RATE AND SEX RATIO AT EMERGENCE OF *G. pallidipes* MAINTAINED ON TPU 2

	Emergence Rate (%)	Sex Ratio
Colony	95.5	1:1
TPU 2-260	92.3	1:1
TPU 2-310	89.6	1:1

there was no difference in both emergence rate and sex ratio at emergence for TPU 2 flies (Table XI).

The large rectangular cages (20 cm × 60 cm × 7 cm high) designed for TPU 2 were shown to cause high mortality and this was thought to be due to an uneven distribution of flies caused by strong light gradient in the cage. Light may attract ("escape" response) or repel flies (resting site selection) resulting in uneven distribution of the flies in the cage. Light may also stress flies by its high intensity, the low flicker frequency of fluorescent tubes or inappropriate wavelength composition. It was discovered that there was significant crowding of flies taking place as a result of the flies being attracted towards the light. Investigations were conducted to determine the effect of light and cage position on survival and production of *G. pallidipes* using both the standard round cage (20 cm diameter × 5 cm high) and the rectangular TPU 2 cages.

Nine round cages were loaded with flies at the standard density of 48 females and 12 males and fixed on the TPU 2 in a 3 × 3 square. Three TPU cages were loaded with flies at the standard density of 260 females and 65 males and placed on the same shelf as the nine round cages. The volume of space /fly in the two cage types was identical. Mortality was checked every week for every cage but PPIF could only be compared between the two sets of cages due to the design of the TPU 2 where pupae could not be collected from each cage separately.

There was a large degree of variation in survival, especially in the three TPU 2 cages. Survival in smaller round cages was better (Table XII). This might suggest that density per volume of cage is not the cause of increased mortality but that the total number of flies in the cage, position or orientation are important. A statistical analysis of the survival data from the

TABLE XII. THE SURVIVAL AND PPIF OF *G. pallidipes* IN STANDARD ROUND CAGES AND TPU 2 CAGES

Survival(%)					
Round Cages			TPU 2 Cages		
41.7	58.3	47.9			
47.9	47.9	35.4	20.4	14.6	48.8
23.1	39.6	37.5			
Mean (%)	42.6		Mean (%)	27.9	
PPIF	2.36		PPIF	1.42	

round cages revealed that there was a significant heterogeneity between the ratios, $\chi^2 = 4.04$, df = 4 and P >0.05 (calculated $\chi^2_{0.05\,(4)} = 4.04$, $\chi^2_{0.05\,(4)} = 9.488$). For TPU 2 cages, the large variation in survival did not permit any meaningful conclusions to be drawn although mean survival was lower than for round cages.

Further investigation was carried out using standard round cages on TPU 2. Twenty seven standard cages (20 cm diameter × 5 cm high) without inserts were loaded with 48 females and 12 males and maintained on the colony regime feeding 3 days a week. Mortality was checked weekly and pupae collected daily. By week 15 the performance of flies maintained in round cages on TPU 2 was PPIF 3.12 and survival 47.07%. For a similar number of cages maintained on the standard colony trolley the PPIF was 3.6.

3.2.2. Discussion and conclusions

An improved version of TPU 2 was evaluated in Ethiopia, Kenya and Tanzania using round cages. In all cases, the mechanical working of the TPU 2 was found to be appropriate and where performance data were obtained, it confirmed that TPU 2 can be used to rear large colonies of flies. But it was also indicated that it was important to have clear knowledge of the physical properties of material from which different components of TPU were made. For example the material from which cages are made should be heat tolerant and be able to withstand the washing process of the dishwasher. Cage washing is carried out in a household dishwasher. We observed that with inserts in TPU 1 cages and TPU 2 cages after a number of washings they would loose shape and with TPU 2 cages could not sit horizontally on the membrane during feeding. It was also observed that the alignment of rods and tension of straps holding cages on the rods was vital for the final fit of cages onto the membrane at the time of feeding and also at rest.

There was a lot of movement of flies in the cage even at rest. The activities appeared to be related to mating activities which take place randomly triggering other flies to respond by flying away. The bigger the cage and therefore the higher the number the flies in the cage, the more the mating activities. Tsetse flies are solitary and may be territorial in their natural habitat. They can therefore be sensitive to all kinds of disturbances and to the population density in the cage. This brings out the possible reason why both survival and production in large cages were poorer when compared to performance of the round cages on TPU 2.

The question of light distribution was resolved by substituting the aluminium with transparent Plexiglas® on the pupal collector in TPU 2. Having established that TPU 2 could be used for

holding and feeding large tsetse colonies and that light distribution could be a major contribution to fly mortality, a further improvement in the handling and feeding of large colonies was the development of the third generation tsetse production unit, TPU 3.

3.3. Tsetse Production Unit 3 (TPU 3)

Unlike TPU 2 where cages containing flies are moved during feeding to the stationary blood source, with TPU 3 blood is moved to flies and the cage holding system is stationary. TPU 3 consists of 5 levels and on each level 9 round cages (20 cm diameter) are held together in a frame (Figs 9 and 10). To solve the problem of light and distribution of flies in the cage, the shelf on TPU 3 onto which larvae drop before crawling into the pupal collector is made of Plexiglas sheets.

The mobile blood feeding system is moved on rails laid on the floor. During feeding all six shelves (of heating plate and trays of blood) are moved simultaneously and raised to make contact with the cages and after feeding, lowered and moved along the rail to the next set of

Figure 9. Prototype tsetse production unit 3 (TPU 3). Two holding units are shown on the left and a feeding unit on the right.

Figure 10. Standard 200 mm diameter cages in a holding frame for the TPU 3 system.

cages. A complete set of the feeding unit has provision to feed at one time two sets of cages in two rows. The blood is heated by elements in aluminium plates individually controlled, instead of the rubber heating mats. Electrical power to the feeding unit is supplied through a spring wound cable drum mounted on the unit. Trays are filled with blood by a person standing on the floor for the lower trays and from a working platform for the upper trays. The working platform is in the middle of the unit with stairs on both sides to step on. There is a temperature sensor attached to one of the heating plates which is connected to the temperature control box that regulates the other plates on one side (max. 5 pieces). For feeding two rows, two control boxes are needed and one heating plate needs between 180 and 260 Watt.

3.3.1. Evaluation

The mechanical evaluation was limited to the ability to move the feeding system along the rails, raising and lowering of feeding trays and heating of blood. Forty-eight female and 12 male *G. pallidipes* were loaded by self-stocking at a ratio of 1 male to 4 females in round cages based on the SSPC system (see Section 4.4). Cages were randomly divided into two groups, one group was put on TPU 3 and the other group was put on the standard colony trolley. Mortality was checked starting two weeks after emergence and thereafter at weekly intervals. Dead flies were counted and 80% taken as representative of females. Flies on the standard trolley were fed three days per week, on Monday, Wednesday and Friday whilst the TPU 3 flies were fed five days per week. Pupae were collected daily (necessary for SSPC) and classified using the pupal sorting machine. Observations were made for 15 weeks and biological data from flies maintained on TPU 3 were compared with data from equivalent

Figure 11. The distribution of faeces on the pupal collection slope demonstrates that adult distribution in the cages is uniform when Plexiglas® pupal collectors are used.

number of cages maintained on the standard trolley. In a second investigation comparison was made between TPU 3, standard trolley and TPU 2 rearing systems.

3.3.2. Results

During the mechanical evaluation of TPU 3 the feeding system was easily moved along the rails and to control movement a mechanical locking device was fitted. Guides were fixed at the corners of the heating plate to enable the cages to fit inside the tray containing warmed blood without further adjustment. In this way once the feeding arm stops under the cage holding frame, all cages would fit precisely on the tray. With no room to include the second feeding arm for a second row of cages in the existing facility it was impossible to fully operate the TPU 3.

The distribution of flies in the cages appeared normal as shown by the distribution of faeces on the shelf on which larvae drop before rolling to the pupal collector (Fig. 11). Observations with TPU 2 had shown a concentration of faeces on the shelf towards the light source.

A larger proportion of flies on TPU 3 started larviposition earlier although the onset, day 20 post emergence, was similar for the two groups. Fecundity (pupae per female per 10 day cycle) P/F/10d was 0.75 ± 0.31 for TPU 3 and 0.59 ± 0.17 for the standard trolley in this trial. The general colony P/F/10d for a similar period was 0.78 ± 0.01. The PPIF was 4.03 for flies maintained on TPU 3 and 4.37 for the standard trolley. The daily mortality was 0.46% and 0.64% for the standard trolley and TPU 3 respectively. The mean colony mortality for the

same period was 0.48±0.08%. The observations indicate that the basic colony parameters for the TPU 3 were comparable to that of the conventional rearing system. Neither overall production nor survival differed significantly between the two groups.

Pupae produced by TPU 3 flies were slightly larger than those produced by the standard trolley flies, but this was not significant. This could have been due to the difference in the feeding regime with TPU 3 being fed 5 days while colony flies were fed 3 days a week. The incidence of soft pupae (pupae which do not form correctly, and die shortly thereafter) was similar for pupae produced by flies maintained using the two systems, 2.66% and 2.55% for TPU 3 and standard trolley pupae respectively.

The comparison between TPU 2, 3 and standard trolley showed no significant difference in pupae per initial female between these systems, although survival of flies on TPU 3 was lower than for the other two systems. Productivity of TPU 2 flies (in standard round cages) was much improved during this investigation as compared to before (Fig. 12). Pupal quality was similar in all the three groups (Fig. 13).

TPU 3 is a further refinement to the TPU series. It would appear that the observed increased mortality associated with light distribution was solved by using Plexiglas pupal collector shelves in place of aluminium sheets. However, the TPU 3 series still uses the standard 20 cm diameter cages. Unless we have reached the end of the road, investigations should continue into developing an optimal cage. Pupal/larval collection is also still carried out manually and efforts should be made to develop a semi-automatic larval/pupal collection system. Note
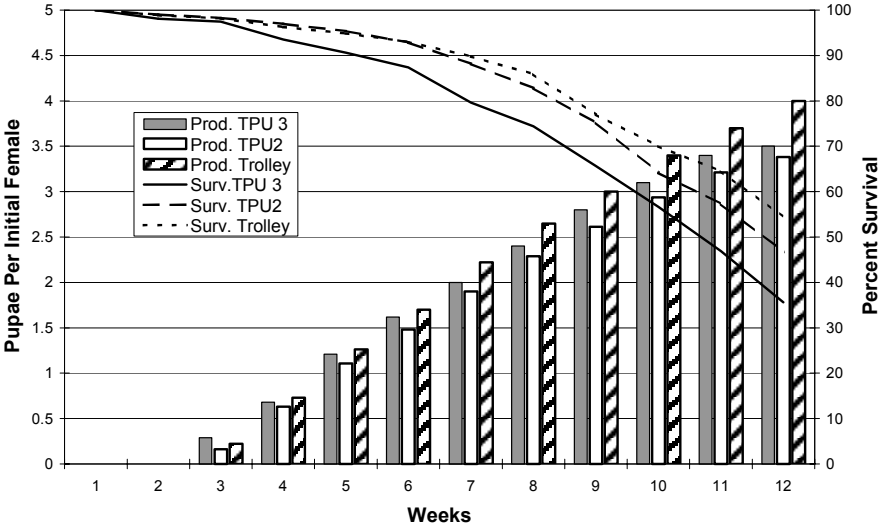

Figure 12. Fecundity and survival for TPU 2, TPU 3 and standard trolleys (control).
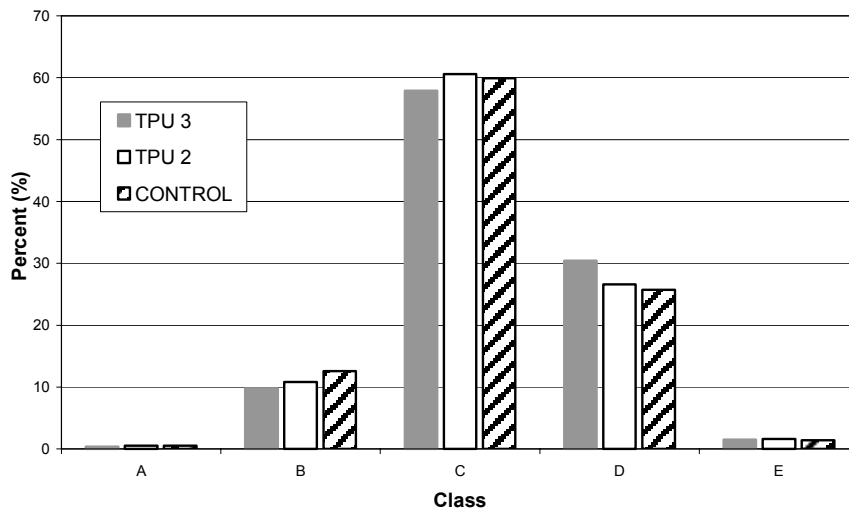
Figure 13. Pupal size classification for TPU 2, TPU 3 and standard trolleys (control).

should be made that larval production continues throughout the 24 hour cycle and the design of the pupal collector should allow pupation of larvae that are produced late during the daily cycle.

All TPU series 1, 2 and 3 continued to use the standard feeding system. Blood being manually loaded on the feeding trays. A design that would allow for a single loading for feeding of the entire row or for replenishment during the course of feeding without interruption would be ideal. The issue of blood quality, the duration of heating and bacterial contamination would need to be investigated. The TPU 3 design have been used in the design of fly rearing facilities in Ethiopia and Burkina Faso.

With an average PPIF of 4.03 for TPU 3 compared with 4.37 for the standard trolley, TPU 3 can be used to maintain a self-sustaining colony.

4. ADULT SEX SEPARATION

During mass rearing tsetse flies are separated according to sex either for loading cages or for processing for release purposes.

At present sex separation in adult flies is carried out manually after chilling the flies at 4°C. The process occurs several times during the life of the fly; before maturity and after mating for females and several times for males. This is the most labour intensive aspect of tsetse rearing, comprising 40% of total labour time. As a result a way was investigated of reducing or eliminating the need for separation of sexes after mating by leaving males in production cages.

4.1. Visual recognition

Initially attempts were made to develop an automatic handling system for sex recognition of emerged flies using an industrial image processing system. The approach was based on the structural texture analysis for the hair distribution on the abdomen. Males have 'hectors' and a hypopygium while females have no distinct features on the abdomen. After defining the distinguishing features of the textured regions a structural analysis of the hair distribution was done. For the recognition of the flies with an image processing system flies had to be presented in an orderly array beneath the camera to ensure correct image capturing. Flies were moved along a track out of a bowl-feeder to a conveyor belt to ensure the right presentation of the fly beneath the camera. It was however, very difficult to position the fly with predefined position (belly-up, belly-down, head forwards, head backwards) and have them at the same distance for image capturing. Because of this, three separate tracks were introduced, one for male, one for female and the third for non-identified flies. The non-identified fly was fed back into the bowl-feeder for a new sex recognition attempt. Flies were immobilised by chilling before presentation.

To achieve individual separation of flies, pressurised air was blown over the fly and the timing controlled by two light barriers (on either side of a tube) and electronic circuitry for signal processing and power supply. The width of the tube was such that only one fly was able to pass through in a longitudinal position. The cooled working environment necessitated that the pressurised air had to be dry and flies could not be exposed for more than 10 minutes to these conditions. It was observed that the power dissipation from the machine and vibration of the feeder affected the overall quality of flies at the end of the process. Finally it was difficult to get the flies in the desired position for the camera to capture the image.

In a later development freshly emerged flies were attracted through the tube towards the camera by light to capture the image. In this set up flies crawled along the tube in the right orientation, head forward and belly down. Images could be captured and used for training the programme, and flies could be separated according to sex. The system still however proved to be slow and unreliable. The computer processing time required to classify an image was around 500 ms, and the system was still sensitive to minor variations in lighting caused by slight variations in the position of the fly's abdomen

One of the drawbacks of this approach was the speed at which separation could be achieved since some flies were reluctant to move soon after they have inflated their wings. UV light was used to attract the flies to move forward but still the whole process was too slow to be of use in a large-scale production facility. The results of the investigations reported above indicated the need to study the behaviour of young adult flies with a view to obtain data that could be used for introducing freshly emerged flies into an automatic handling system of an image processing device for sex recognition. The main mission was to make freshly emerged flies to enter a narrow passage in which they are recorded. Since flies are attracted towards light during the first few hours of adult life, the onset and peak of positive photo response of freshly emerged flies towards UV and white light were studied. It was observed that newly emerged flies went through 3 distinct behavioural stages:

- Early dispersal of non-expanded imagoes with erratic movements lasting only a few minutes.

- Expansion phase approximately 20 minutes during which the fly remains motionless and attains final size and shape.

- Post-expansion dispersal, photo-positive behaviour.

A photocell recorded photo responses of flies to UV and white light and signals were collected by a computer and the number of flies responding within 10 minute intervals recorded. In later investigations only white light was used since results showed that white light attracted the flies as well as UV light. When expanded flies aged for 30 minutes in the dark were exposed to light, they began to respond 50–70 minutes after transfer to light. One half responded within 100 minutes. Most flies responded within 6 hours and response was similar for the two species of tsetse investigated, *G. pallidipes* and *G. austeni*.

In summary, freshly emerged flies could not be manipulated by light before they have expanded their body and wings and completed cuticular tanning. This process takes more than one hour. After this process the photo response was weak and delayed and cannot be controlled. The response was too slow to use for driving the imagoes into the sexing apparatus and hence the behaviour cannot be used for manipulating young teneral flies for sex separation in mass rearing facilities.

4.2. Adult sex separation based on development time

While investigations on mechanical sex separation using visual characteristics was going on Žďárek and Denlinger [13] suggested that the differences in development rate between sexes of tsetse are of such a nature and extent that they could be exploited for automated separation of sexes. They found that the timing of eclosion is a response driven by the circadian changes of environmental temperature and not by photoperiod. Experiments were designed to study the difference between emergence of females and males in *G. austeni,* to determine the conditions for synchronisation of emergence time to give a clear separation of sexes and to find the appropriate cage in which the freshly emerged flies would easily be collected and quality assured.

G. austeni pupae collected within a 24 hour period were incubated under normal conditions until one day before the first flies began to emerge. At this point in time the puparia were subjected to fluctuating temperature in an emergence apparatus that automatically recorded eclosion time of individual flies within 30 seconds. The sex ratio of emerged adults was checked at regular intervals of 3 hours during the day for the whole duration of emergence. Flies were allowed to emerge from crowded pupae either under fluctuating temperature or under constant temperature of the incubation room. In an attempt to synchronise initial female emergence, batches of *G. austeni* from which the first pioneers had emerged were transferred to several low temperatures ranging from 10 to 18.5°C for 2 to 4 days. The pupae were then moved to 26.5°C and their emergence and sex ratio recorded daily.

4.2.1 Results

Adult emergence of *G. austeni* showed a regular circadian rhythm that is driven by the diurnal temperature cycle. The time of emergence can be experimentally manipulated by changes in temperature while photo period has no effect on emergence rhythm. Males and females show differences in the rate of their intrapuparial development. The females have a faster rate and consequently emerge earlier than the males deposited during the same day. Females emerge

approximately 2 days earlier than males at incubation temperature of 24–25°C. The emergence period spreads over a 4–6 days period depending on temperature. At 24, 25 and 26°C the emergence periods lasted 6, 5 and 4 days, respectively. Only females emerged during the first days and mostly males emerged during the last days. The sexes were mixed in the middle of the emergence period [13].

Fluctuating temperature gave no significant improvement in the accuracy of sex separation. Better separation was achieved when pupae were not in contact with each other than when allowed to touch during emergence. It is known that mechanical disturbance will induce emergence, so it was thought that emerging flies disturbed adjacent pupae which disrupted the emergence pattern.

4.3. Discussion

One of the major constraints for automation of tsetse fly mass rearing technology has been the lack of a reliable technique for sex separation. The freshly emerged flies have to be manually sorted after being chilled, which is a labour intensive procedure that may lead to a possible latent damage to the chilled insects and may present health problems to the personnel. In order to overcome this technological bottleneck more efficient methods have long been sought. The recent investigations on the differences in the rates of intrapuparial development of females and males of tsetse flies causing females to emerge 2–3 days earlier than males have laid the grounds for designing a technique of sex separation appropriate for and application to automated mass rearing facilities.

Since the emergence rhythm is dependent on daily temperature changes, it is not surprising that manipulation with ambient temperature before and during emergence period was found to be a useful tool for increasing accuracy and predictability of sex separation based on differential emergence of males and females. The conditions under which the emergence periods of females and males were as short as possible and with least overlap was 26.5°C. At this temperature, the overall emergence period for pupae collected within a 24 hour period was shortened to 4 days with females appearing during the first two days. In order to shorten further the emergence period of the earlier emerging sex, the females, low pre-emergence temperature exposure of pupae for varying periods were tested with the aim to find conditions that would allow completion of development and inhibiting the onset of emergence. The requirements that met these conditions were 18.5°C followed by emergence at 26.5°C. Under these conditions the overall emergence period was compressed to just three days with 75% of

females emerging on the first day. But there was increased mortality among flies emerging from pupae treated this way.

The accuracy of sex separation based on differences in emergence time of females and males can be enhanced further by keeping pupae separately during emergence and not allowing them to touch each other. The pupae from the mother colony should be collected at the same time everyday and should be incubated under constant standard incubation conditions irrespective of photoperiod.

4.4. Self-stocking of production cages (SSPC)

The above differences in emergence time were developed for self-stocking of production cages. Flies were made to emerge directly into production cages in the correct number and sex ratio eliminating the need for chilling and manual separation of sexes; a step which takes 23% of time invested in tsetse mass rearing. Using the established pupal emergence rate, the number of pupae required to load the cage with the desired number and sex of flies is calculated assuming an initial equal proportion of male and female pupae. In nature larvae burrow into the ground where they pupate and after the development period, the operculum opens and the young fly with unexpanded body and wings works its way to the surface of the soil. This behaviour combined with the fact that freshly emerged flies penetrate the netting of the standard holding cage (provided the netting rests on the pupae) led to the development of the SSPC.
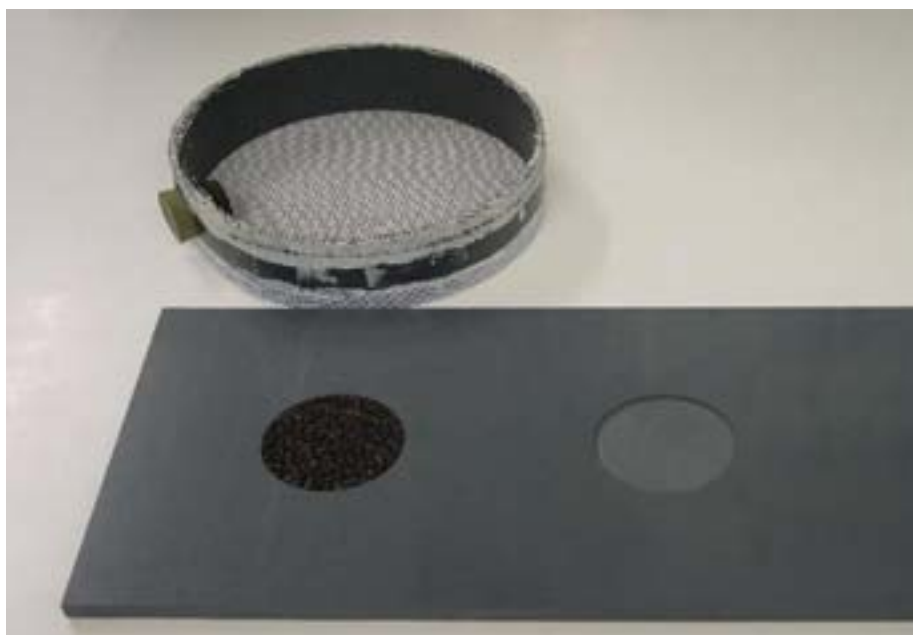

Figure 14.   Pupal emergence system with milled cavities to hold the pupae under a cage. The cavity diameter is adjusted so as to hold the correct number of pupae to stock one cage to avoid the need to count pupae.

Figure 15. In another version, pupae were held in a rubber "O"-ring under the production cage. The large mesh white net can be seen on the bottom of the cage over the pupae; the teneral adults can pass through this mesh but not the smaller mesh on the top surface.

4.4.1. G. austeni

To test the SSPC for *G. austeni* with the aim of stocking the cages with 100 females and 25 males, pupae collected daily were incubated in open trays at 23–24°C and 80% RH for 25 days. After this period, the pupae were placed in Petri dishes under a standard emergence cage until the first fly emerged. At this point the pupae were moved to 26.5°C and placed in a single layer under the production cage (Figs 14 and 15). The cage was removed at 24, 48, 72 and 96 hours and the number of flies in the cage counted. It was observed that the critical period was between 48 and 72 hours so in the next series of experiments cages were removed hourly between 9:00 and 15:00 on those days. The collection at hourly interval indicated when males started to emerge and the actual time when the required number of males emerged. Having established that the cages would be stocked with the right number of flies and sex ratio, cages stocked this way were observed for pupal production and survival and compared with performance of flies manually stocked with 100 females and 25 males.

At 23–24°C emergence was completed after 6 days (Fig. 16) while at 26.5°C, emergence was reduced to 4 days (Fig. 17). During the first two days, predominantly females emerged and the last two days males. Males started to emerge during the second day and 52 hours from the onset of emergence, production cages were stocked with about 100 females and 25 males, a 1:4 male to female ratio (Fig. 18). Production at week 10 for flies emerged using the SSPC was PPIF 3.39 compared to 3.67 for manually loaded cages. Pupae remaining after cages have been stocked were predominantly male pupae and could be irradiated and processed for release without undergoing chill separation. The sexing error for males destined for release would be on average less than 0.5%. For comparison the recorded rate of sexing error for the
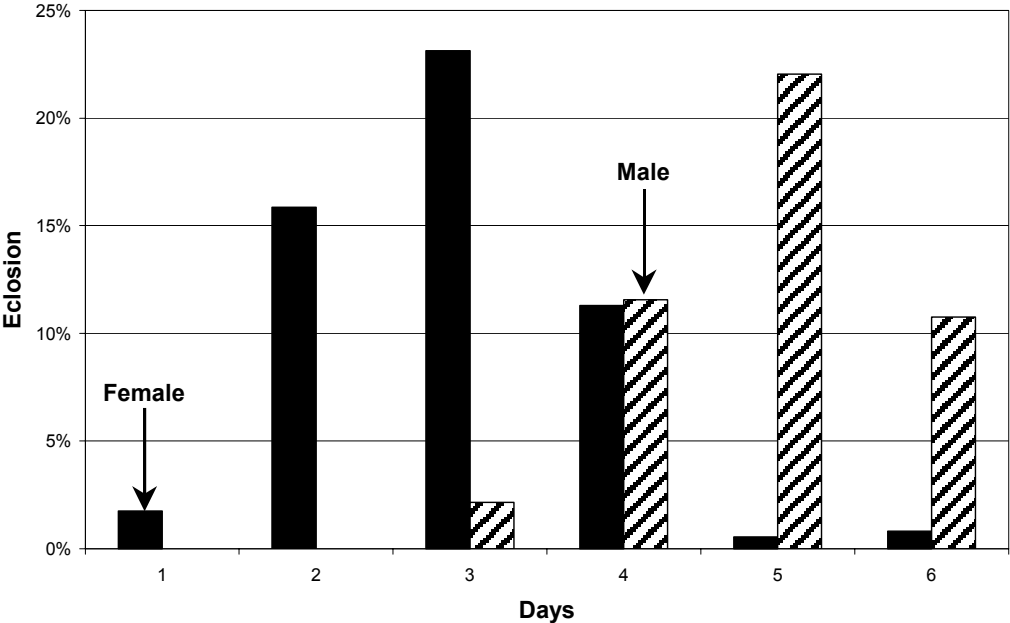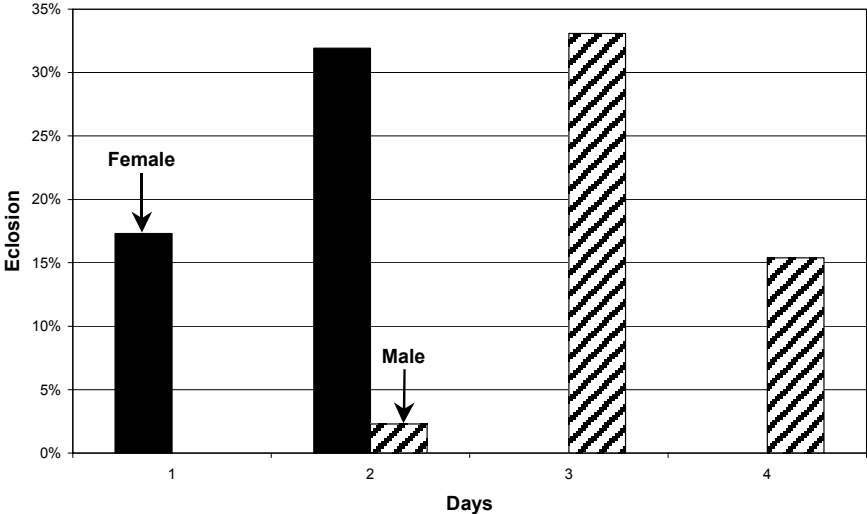

Figure 16. Emergence pattern of G. austeni at 24°C.
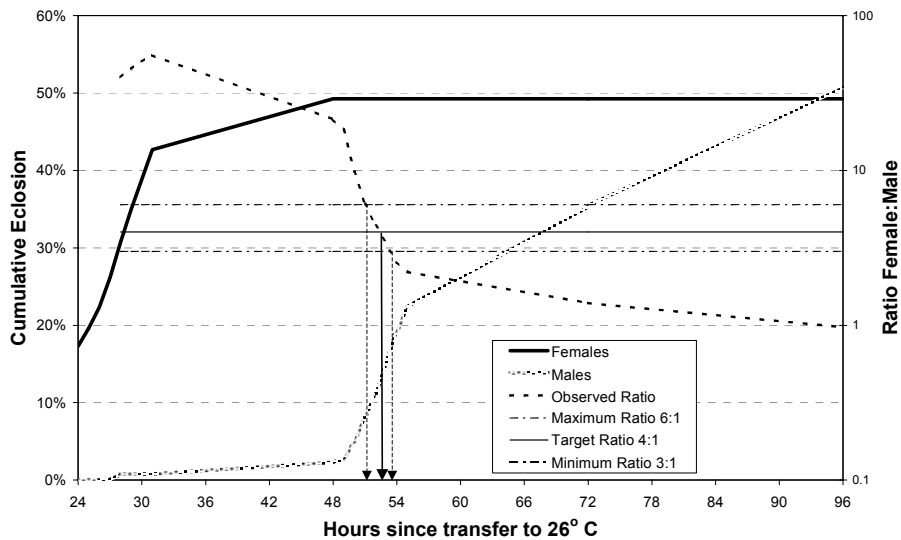

Figure 17. Emergence pattern of G. austeni at 26°C.

Figure 18. Sex ratio of G. austeni at emergence at 26°C.

manually sexed flies for the Zanzibar eradication was approximately 1.2%. Elimination of chilling and separation reduces the time invested in handling young flies and together with Day 0 mating and resident males used for mating, the total amount of time required for tsetse rearing is reduced by more than 40%.

4.4.2. G. pallidipes

After demonstrating that with *G. austeni*, production cages could be loaded at the time of emergence using the SSPC procedure, tests were carried out with *G. pallidipes*.

Pupae collected on a daily basis were incubated at 23–24°C for 25 days after which they were transferred to Petri dishes and kept below special emergence cages. When the first flies emerged about 31 days after larviposition, pupae were counted into batches of 110 and divided into two groups. This is the number of pupae that would give rise to 48–50 females at emergence.

One group was kept at the same pupal incubation conditions but in a single layer in a ring below the production cage and the second group was taken to a room set at 26.5°C and RH 75–80%. Flies were allowed to emerge directly into the production cages and counted on a daily basis when the monitoring cages were replaced. From the onset of observations it was found that unlike with *G. austeni* where enough males emerged together with the females by 52 hours after transfer to the higher temperature, the number of males that emerged were not adequate for loading the cages. A system was then devised to place together 110 pupae and 15 'male' pupae to stock a 20 cm diameter cage. The 'male' pupae were obtained from residual
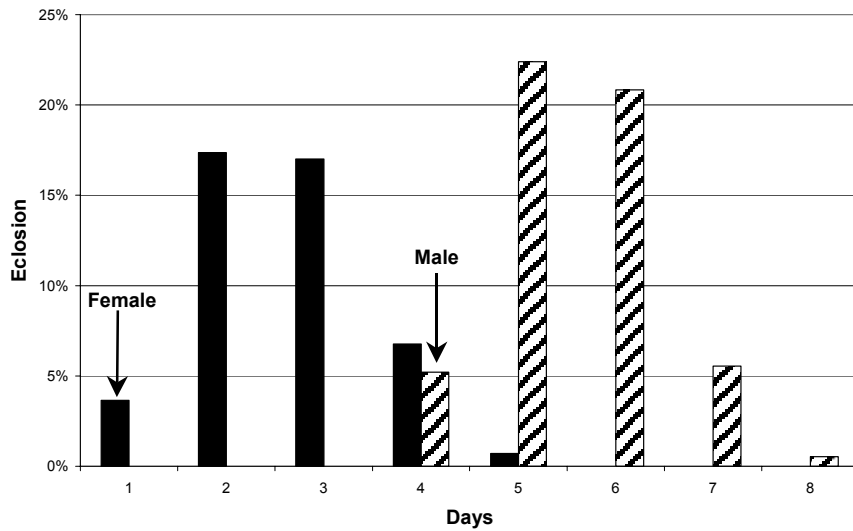
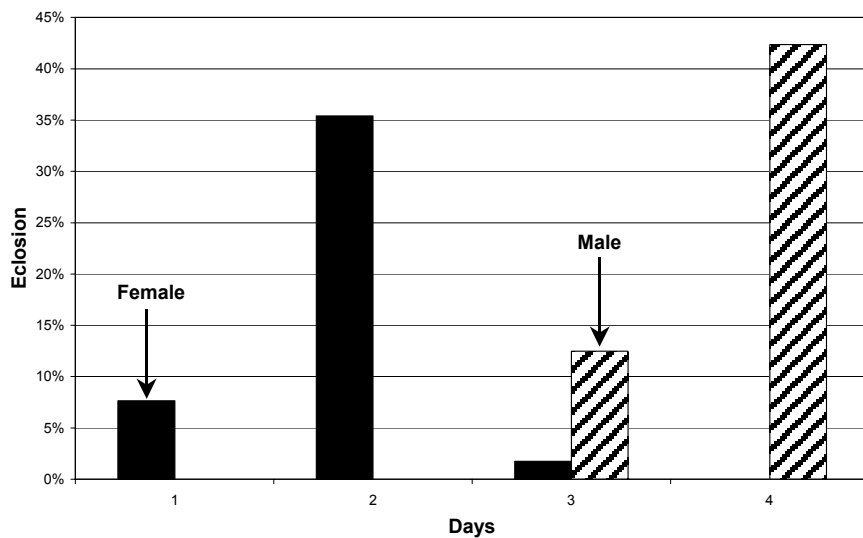*Figure 19. Emergence pattern for G. pallidipes at 24°C.*

Figure 20. Emergence pattern for G. pallidipes at 26.5°C.

pupae after all the females had emerged into production cages. This way enough males would emerge together with the females and at 48 hours from the time mature pupae are transferred to the higher temperature, the cages would be stocked with the desired sex and number of the flies. The residual pupae would provide 'male' pupae for the next batch of mature pupae.

At normal holding conditions of 23–24°C and 80–85% RH, emergence of *G. pallidipes* pupae deposited on the same day span over a 7–8 day period. During the first three days only females emerge and males emerged over the last four days (Fig 19). When pupae ready to emerge were transferred to 26.5°C, the emergence period was reduced to 4 days. Almost all the females emerged during the first two days and males emerged during the last two days (Fig. 20). This allows for a separation of sexes but without males required for mating colony

TABLE XIII.   AVERAGE NUMBER OF *G. PALLIDIPES* FLIES THAT EMERGE PER DAY WHEN MATURE PUPAE TOGETHER WITH MALE PUPAE ARE MOVED TO 26.5°C

Emergence day	♀♀	♂♂	Total	
			♀♀	♂♂
1	18	6	-	-
2	30	7	48	13
3	3	18	-	-
4	-	26	-	-
5	-	3	-	-

females. On day 3 and 4 the emergences are almost all males and when the required number of these pupae are put together with mixed pupae at the start of the cage stocking, the cage would be stocked with the right number and required sex ratio for colony mating by 48 hours (Table XIII). 110 mixed and 15 "male pupae" are required for loading a cage with on average 48 ♀♀ and 13 ♂♂ in 2 days giving a ratio of 1 ♂♂ to 4 ♀♀. The data was collected from 20 replicates.

From the investigations using two species of tsetse it was shown that cages can be stocked directly with the right number of flies at the desired sex ratio without need for chill separation. There are several advantages to this procedure. First of all the procedure simplifies and eliminates the most labour intensive part of tsetse rearing and only requires that temperature in the incubation and emergence rooms be kept constant. Secondly, it reduces the handling of young flies thus minimising damage that may occur during handling and improving the quality of the flies. In addition the system can be used for production of male only pupae.

5. GENERAL DISCUSSION

The tsetse production unit was developed to provide a system that would hold a large number of flies in cages and bring them automatically for feeding while pupae are collected centrally. First and foremost the mechanical developments had to be matched to the biological requirements. Tsetse flies are known to be solitary organisms and aggregate only on hosts during feeding when males seize the opportunity to inseminate the females. After engorging with blood, a fly seeks a dark place usually the underside of a branch and sits to digest the blood until it is hungry again and requires another blood meal. It is estimated that a fly seeks a blood meal every three days. During the period digestion is taking place movement is limited only to seeking a suitable habitat or when disturbed and for females looking for a suitable larviposition site. A tsetse fly therefore is active for an estimated 15 minutes a day. The design of the TPU 1 enabled cages to be automatically fed and the feeding lasted 3 hours. After the feeding the cages were kept in motion to enable all cages to experience the same climatic conditions over the remaining 24 hours. The high daily mortality recorded was shown to be due to movement when survival of flies in cages kept without movement was compared to those maintained on TPU 1. It was also observed that most of the dead flies were starved, an indication that either feeding was inadequate which was proved otherwise or flies were using up too much energy before the next feed as a result of the continuous movement. As for the inserts that could increase the carrying capacity of cages, experience with cardboard inserts was excellent though impractical but the plastic inserts did not perform as well and were thereafter discontinued.

Having established that movement was detrimental to fly survival and that the design was not suitable for rearing tsetse under African conditions, a simplified prototype TPU 2 was developed and evaluated. TPU 2 assumed that all operations would be hand powered and only the heating of blood would require electricity.

The design of cages for housing tsetse generally fulfils the following functions; retaining flies inside the cage while allowing for feeding to take place and larvae to crawl out. In later developments the netting had to be large enough to enable newly emerged flies to get into the cage during self stocking but at the same time small enough to retain them before they expands their wings. The TPU 2 cage therefore was covered by fine netting on the upper side and a larger netting on the side that sat on the pupae. Inserts in TPU 2 cages were replaced with benches/shelves on which flies would perch in addition to the netting on either side of the cage and the vertical walls. The cages were larger than the standard cage and housed many

more flies. The concept of feeding large number of cages automatically was demonstrated but the TPU 2 still failed to meet the requirements of maintaining a self-sustaining colony. The major drawbacks were the cage design. In later investigations the standard round cage was used with TPU 2 and when the Aluminium shelf was replaced with the transparent Plexiglas performance of flies on TPU 2 was comparable to that of the standard trolley used in conventional rearing. The positive features from TPU 2 were used to design TPU 3. The main difference between TPU 2 and TPU 3 is that in the latter the frame holding the flies is static, and the blood is brought to the cages on a special trolley mounted on rails. Comparison of performance of flies on TPU 3 with those on TPU 2 with Plexiglas shelves and the standard trolley confirmed that a semi-automated tsetse production unit capable of maintaining a self sustaining colony was available.

A mating regime appropriate for automated mass rearing and direct loading of production cages was developed. The mating system using resident males was initially tested with *G. austeni* using ratios of 1 male to 4 females and was shown not to affect survival and production. The procedure has now been tested with other species kept at the Seibersdorf laboratories. The current mating regime for all species utilizes a sex ratio of 1 male to 3 or 4 females depending on species. The revised procedure saves 17% of time invested in tsetse mass rearing and removes one chilling procedure that was necessary after mating.

Day 0 mating with resident males using predetermined sex ratio was shown to be effective. The procedure involves putting together teneral flies of both sexes in production cages in the right sex ratio at the time of emergence and leaving them permanently together. Mating takes place as the flies mature over the first 5–10 days. This procedure was adopted for routine maintenance of colonies at TTRI and Seibersdorf.

Adult sex separation of teneral flies based on the differences in emergence time was demonstrated and tested in Ethiopia, Burkina Faso and Tanzania. Seibersdorf has adopted the procedure for *G. pallidipes* (UGA) colony. Combining sex separation by timing of emergence, direct emergence into production cages and resident males has completely removed the need for chilling for sex separation. This system may be used for production of male pupae only or sterile male production.

Three prototype tsetse production units (TPU) for holding and feeding of colony flies and larval collection were designed, constructed and evaluated.
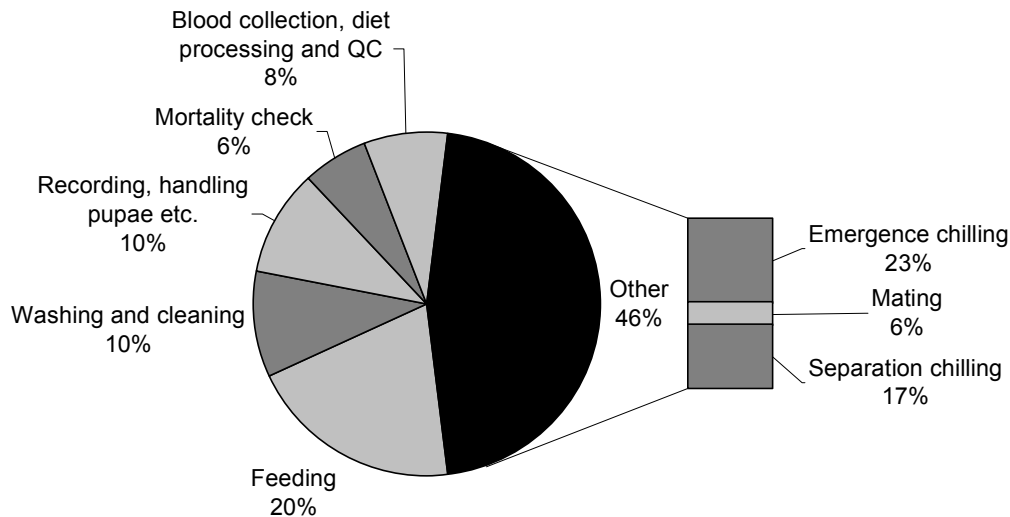
Figure 21. *Reduction in the labour involved in tsetse colony rearing achieved during the course of the Co-ordinated Research Project. Removal of all chilling reduces handling by 46%, and automation of feeding achieves a further reduction of about 15%.*

TPU 1, a prototype that held fly cages, automatically moved cages to the blood source for feeding while larvae and pupae were collected centrally was the first prototype evaluated. The evaluation however, revealed that the prototype was incapable of maintaining a self-sustaining colony of *G. austeni*. Experience gained lead to the development of a second prototype, TPU 2, a simplified version that could be locally constructed and serviced.

TPU 2 prototypes were evaluated in Ethiopia, Kenya and Tanzania, for mechanical characteristics and suitability for maintaining colony flies. Although TPU 2 proved suitable for maintaining a colony of tsetse, it was observed that the distribution of light inside the cage was important. Subsequently, the aluminium shelf pupal collector was replaced by clear Plexiglas. Since movement was demonstrated to be detrimental to fly performance a further design TPU 3 was constructed and evaluated.

In TPU 3 fly cages are held stationary and blood in the feeding system moved on rails is taken to the cage holding frame, raised to make contact with netting of the cages during feeding and thereafter lowered and moved to another set of cages. Two rows of cages are fed at once. TPU 3 is very satisfactory and is comparable with the standard rearing system in terms of production and adult survival.

The development and evaluation of mechanical components of hardware for tsetse production units led to the improvement of holding and feeding systems appropriate for use by large

colonies. Physiological and behavioural studies to understand the target organism contributed to the formulation and design of automated sex separation and loading of production cages.

The Co-ordinated Research Project has resulted in:

- the development/adaptation of ancillary equipment e.g. sterilising oven and aluminium heating plate for use with the semi-automated holding and feeding system.

- Improvement in young fly handling, eliminating labour intensive steps equivalent to about 50% of all manual labour (Fig. 21) and therefore cutting back on cost of rearing and enabling standardization of quality sensitive steps.

Based on the results of the CRP, a model tsetse production facility was designed for the eradication project in Ethiopia and upgrading of facilities in Burkina Faso and Uganda are under way.

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