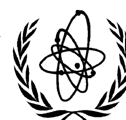

ENTOMOLOGY UNIT,
FAO/IAEA AGRICULTURE
AND BIOTECHNOLOGY LABORATORY,
SEIBERSDORF

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1. INTRODUCTION

The framework within which the Unit carries out its assignment is the Insect and Pest Control Sub Programme D-4, the cornerstone of which is the development and implementation of the Sterile Insect Technique (SIT). The Unit supports the sub-programme by carrying out R&D for the medfly, *Ceratitis capitata* and tsetse in support of operational projects, providing services to counterparts in Member States and providing training for fellows. The highlights of work carried out by the Unit in 1997 include consolidation in the use of medfly genetic sexing strains (GSS) in operational SIT programmes, the elaboration of a new cage stocking procedure for tsetse and the production of transgenic medflies. The details of these highlights can be found in the body of the report but they will be summarized here.

Medfly GSS are now being reared in five large facilities (Central and South America and Portugal) to supply males only for operational SIT programmes and future programmes in the Middle East and South Africa will also use this technology. The introduction of a recombinant filter system (described in Annual Report 1996) in these facilities has given programme managers a flexible tool with which to maximise the potential of sexing strains. There is a constant evolution of these strains based primarily on the experiences gained during their large scale mass rearing, a process that cannot be carried out at Seibersdorf. The knowledge gained during mass rearing is fed back into the development part of the project and improvements realized. The enthusiasm with which programme managers embrace this new technology indicates the benefits they receive from the use of GSS for their field programmes.

The status of tsetse mass rearing, relative to that which has been developed for other SIT targeted pests, is the major constraint for the wider application of this technique for tsetse. Fly handling procedures have to be reduced in number and scope to enable a more economic production of flies to be accomplished. Research in the Unit has demonstrated that many procedures can be simplified without compromising fly production and quality. Several of these procedures were transferred to the Tsetse and Trypanosomosis Research Institute at Tanga to enable the *G. austeni* colony to evolve to a size where very large numbers of sterile males could be released in Zanzibar leading to the eradication of the fly on the island.

The strategic development of genetic sexing strains in medfly will depend on being able to introduce DNA into the medfly genome and transgenic techniques are therefore essential. Although the development of genetic transformation technology is not within the mandate of the Unit, the unique place that the Unit has created for itself in this field makes it indispensable to the practical application of this technology. The Unit frequently hosts collaborators who wish to carry out experiments on medfly in this area and in 1997 the first successful transgenic medflies were produced. These strains are currently being studied for aspects related to their use in future applied studies.

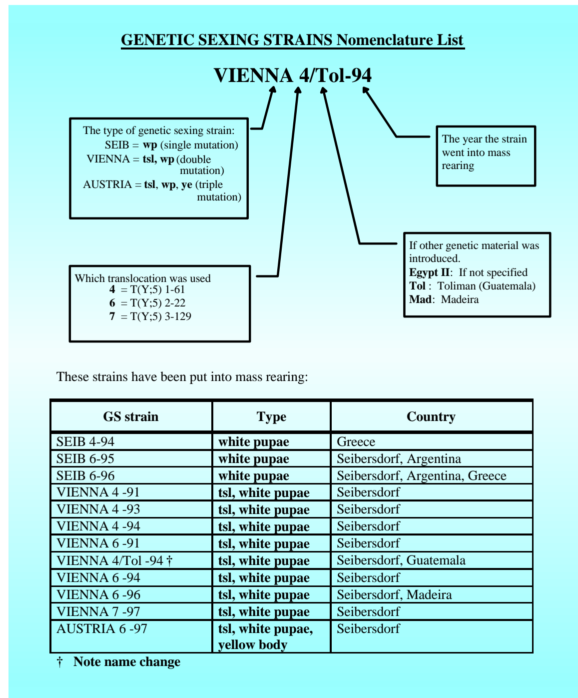
There was disappointment in that the results from the evaluation of the Tsetse Production Unit (TPU) led to the decision to discontinue work on this prototype. Major faults in cage design based on a flawed concept caused excessive fly mortality. This highly automated experimental holding and feeding system for tsetse flies was shown to be incapable of maintaining a self-sustaining colony of *Glossina austeni*. As the machine was impossible to modify without major changes in construction, the evaluation was terminated. A number of technical improvments emerged as a result of this evaluation but, the major lesson learned from this experience is that a too highly automated and inflexible design does not provide the kind of basis to evolve an improved system for tsetse rearing. A second simpler rearing system is currently

being tested which incorporates some of the positive developments apparent from the evaluation of the TPU.

1.1. SERVICE TO MEMBER STATES

The Unit is now the acknowledged world center for medly genetic diversity and it is also the only laboratory which can supply clients in Member States with tsetse flies from six different species. The unit currently maintains some 70-80 specific medfly genetic strains together with about ten wild strains from various parts of the world and six different tsetse species. The maintenance of this biological diversity and its distribution to the Member States provides the unit with a pivotal role in the support of many varied R and D projects on these insects. For tsetse, many collaborators depend totally for their research on the supply of material they receive from Seibersdorf. Colony and strain maintenance is generally not regarded as a particular glamorous activity but it fulfils an essential role and fits well with the international mandate of the Agency. The Unit also supplies quality tested irradiated frozen bovine blood for those collaborators who cannot find a suitable source of blood for *invitro* tsetse rearing.

An important area in which the Unit services the needs of Member States is in the transfer of medfly genetic sexing strains to large SIT field programmes and the provision of subsequent technical support. The transfer of the strains is followed up by continuing advice and interpertation of the behaviour of the strains under the diverse rearing conditions at different facilities. The supply of the total package, the strain plus the recombinant filter and continued support is essential to ensure client satisfaction.



These strains have been put into mass rearing:

GS strain	Type	Country
SEIB 4-94	**white pupae**	Greece
SEIB 6-95	**white pupae**	Seibersdorf, Argentina
SEIB 6-96	**white pupae**	Seibersdorf, Argentina, Greece
VIENNA 4 -91	**tsl, white pupae**	Seibersdorf
VIENNA 4 -93	**tsl, white pupae**	Seibersdorf
VIENNA 4 -94	**tsl, white pupae**	Seibersdorf
VIENNA 6 -91	**tsl, white pupae**	Seibersdorf
VIENNA 4/Tol -94 †	**tsl, white pupae**	Seibersdorf, Guatemala
VIENNA 6 -94	**tsl, white pupae**	Seibersdorf
VIENNA 6 -96	**tsl, white pupae**	Seibersdorf, Madeira
VIENNA 7 -97	**tsl, white pupae**	Seibersdorf
AUSTRIA 6 -97	**tsl, white pupae, yellow body**	Seibersdorf

† **Note name change**

1.2. GENETIC SEXING STRAIN NOMENCLATURE

The mass production of GSS around the world has necessitated a common nomenclature and this is shown above. This nomenclature is only used for GSS which have been mass reared and strains which are still under development or are of purely an experimental nature are not always named with the same amount of detail but in general this nomenclature is used throughout the report

1.3. CONSULTANTS AND FELLOWS

Consultancies are a very efficient mechanism to advance the work carried out in the Unit and four scientists contributed their expertise in 1997, two in tsetse and two in medfly (see Table 1.1). The medfly consultants focused on the development of transgenic techniques and the cytological analysis of GSS. Dr Handler from the University of Florida was successful in producing transgenic medflies, which are now being analysed in the Unit for characteristics which are important for their future application. Dr Zacharopoulou from the University of

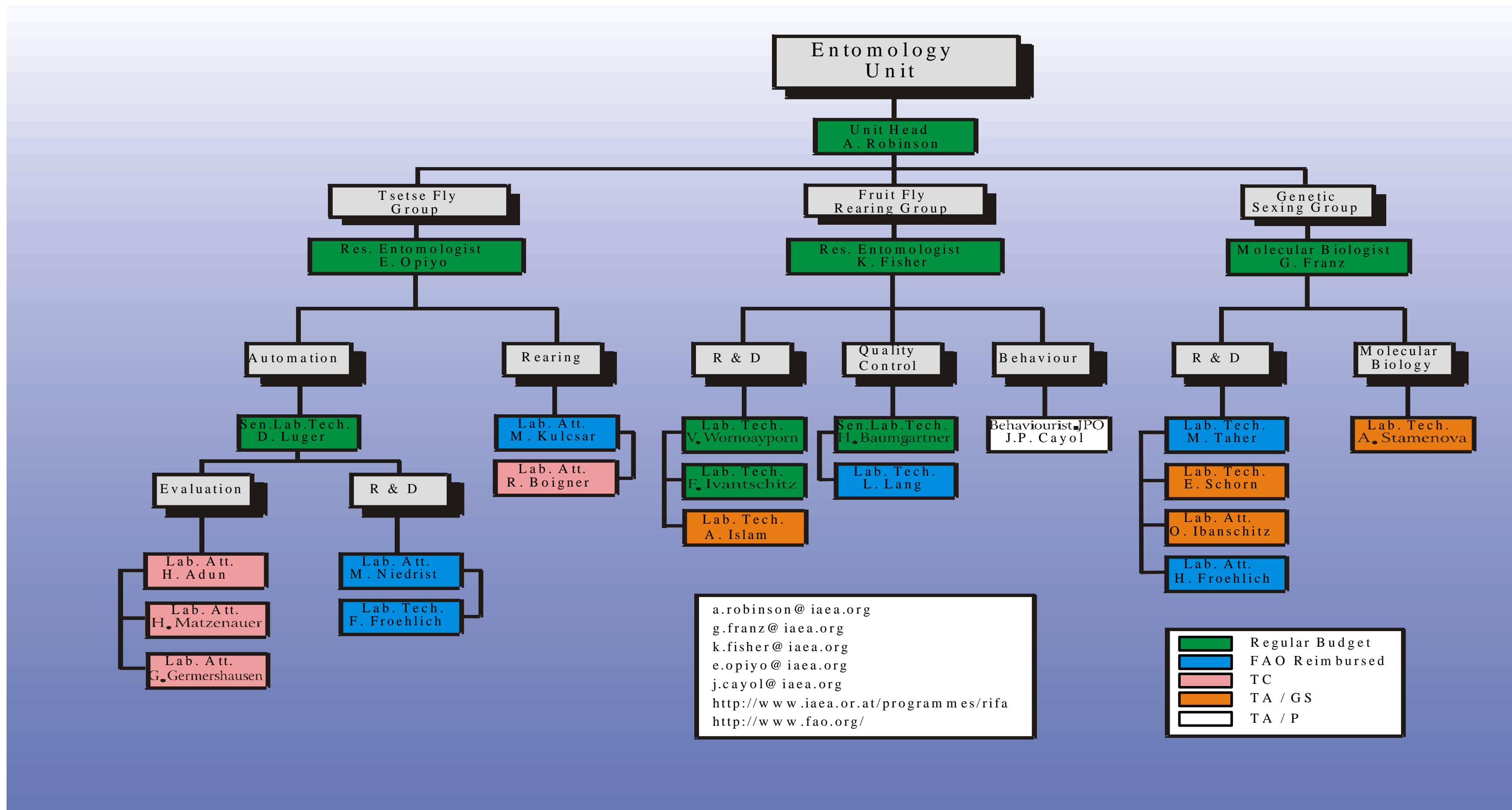
Patras conducted a cytological analysis of medflies from the GSS being reared in Mendoza, Argentina and she was able to confirm that a particular type of genetic recombination was occurring. In tsetse a new and potentially very sensitive population analytical technique was introduced by Dr. Krafsur, University of Iowa. The technique is able to analyse single base pair changes in DNA. Cytoplasmic incompatibility is a natural form of sterility often found in insects and Dr Bourtzis of the University of Yale tried unsuccessfully to infect *G. pallidipes* with a bacterial symbiont that can cause this phenomenon. Studies carried out by consultants complement but do not duplicate work being carried out in the Unit.

The number of fellows trained in 1997 was much lower than in previous years with only a total of 2.35 man/months utilized.

Table 1.1: Consultancies carried out in the Unit in 1997

Dr. K. Bourtzis	1997-06-12 - 1997-06-27	University of Yale
Dr. A. Handler	1997-11-03 - 1997-11-12	University of Florida
Dr. E. Krafsur	1997-09-22 - 1997-10-03	University of Iowa
Dr. A. Zacharopoulou	1997-04-07 - 1997-04-16	University of Patras

1.4. ORGANOGRAM



2. TSETSE GROUP

The emphasis in the group remains the improvement of tsetse fly production both in terms of cost and logistics. Some of the developments reported in the 1996 Annual Report were transferred to the Tsetse and Trypanosomosis Research Institute at Tanga and they enabled the *Glossina austeni* colony to be expanded to a level of almost one million females resulting in the release of up to 110,000 sterile males/week on Zanzibar. This level of release caused the wild population to collapse and resulted an indepenedent experts group confirming the eradication of tsetse.

The proposed SIT component of a tsetse eradication programme in the Southern Rift Valley in Ethiopia will require a major change in fly production methodology as well a move to a different species, *G. pallidipes*. Many of the improvements developed for *G. austeni* will have to be re-assessed for the new species and a start was made in this during 1997. To support an SIT component for the programme in Ethiopia, the group will need to work in two areas, the first and most important being that of rearing technology improvement since upscaling of the procedures from Tanga will not be appropriate. The second area relates to the genetic analysis of the target *G. pallidipes* population to establish population structure and isolation. This type of analysis is essential in order to design the location and size of barriers to fly movement in order to maintain a fly free area. Both these studies are supported by an Agency Co-ordinated Research Programmes.

2.1. EVALUATION OF THE TSETSE PRODUCTION UNIT (TPU)

The evaluation of the TPU was carried out using *G. austeni* as this colony could provide the required number of flies. During 1996 the performance of flies on the TPU was found to be disappointing

with many flies dying at a very young age and a series of factors were assessed as to their effect on survival and some changes were introduced (see Annual Report 1996). By the end of 1996, some improvement in survival was noticed encouraging the goal of achieving a self sustaining colony on the TPU in 1997. This report includes information on performance of five generations of flies which were kept between the period August 1996 and August 1997. Two main indicators were used to determine the performance of flies namely; survival and pupal production. Because of the specific shortcomings in 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. The inability to collect accurate data on important biological parameters was a major design fault in the machine.

Modifications introduced during 1997 included cages with half inserts and five instead of six day feeding per week with extended feeding time on Mondays and Fridays. In September, 1997, flies from the TPU were moved as the building housing the TPU had to undergo a major structural repair. Cage types and the orientation in which cages were held were temporarily changed for six weeks and thereafter the flies were returned to TPU cages in a vertical orientation simulating the orientation of the cages on the TPU but they continued to be manually fed.

Table 2.1: Calculated performance of TPU flies compared to that of colony between July 1996 and June 1997

Period	Input	% Daily Mortality		No. of Pupae	P/F/10d	
	TPU	TPU	Colony	TPU	TPU1	Colony
Jul-96	19119	1.26	0.86	10185	0.41	0.83
Aug-96	8948	2.77	0.81	9013	0.52	0.92
Sep-96	19453	0.91	0.85	12820	0.49	0.98
Oct-96	23549	2.81	0.66	15515	0.53	0.74
Nov-96	22009	6.19	0.44	13480	0.45	0.84
Dec-96	15480	2.19	0.51	18151	0.73	0.80
Jan-97	27604	4.31	0.43	13249	0.39	0.77
Feb-97	6031	1.94	0.33	25048	1.37	0.80
Mar-97	14134	1.73	0.55	18503	0.96	0.82
Apr-97	7731	5.25	1.54	11727	0.82	0.87
May-97	9070	3.63	1.24	8531	0.64	0.95
Jun-97	7615	1.66	0.95	10706	0.91	0.98
Total	180743	34.65	9.17	166928	8.22	10.3
Average		2.89	0.76		0.69	0.86

Table 2.2: Size classes *G. austeni* pupae (%) by generations in July 1997

Generations	A	B	C	D	E
G0	3.2	29.9	53.5	11.8	1.6
F1	7.4	23.5	60.3	7.4	1.5
F2	8.6	31.4	54.3	5.7	0.0
F3	7.7	27.5	51.6	14.1	0.0
F4	3.3	16.7	62.6	13.2	2.2
F5	2.8	29.2	45.8	16.7	5.6
COLONY	2.1	24.0	62.8	11.0	1.0

(Columns A–E: Classes)

A large number of flies was introduced onto the TPU (Table 2.1) but it was not possible to obtain a self sustaining colony. This was due almost entirely to the high percent daily mortality (mean 2.89%). The mean daily mortality was 0.76% for a similar period among flies maintained in the routine colony. In the period, July 1996 to June 1997, 180,743 flies were added to the TPU with only 166,928 pupae being produced.

The fecundity of flies that survived was 0.69 pupae/female/10 days (P/F/10d) for TPU 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.

The quality of pupae produced on the TPU was inferior to those produced in the normal colony (Annual Report, 1996). This could have been due to the way they were handled and to the physiological status of the female flies. Having terminated the continuous movement of cages which had been shown to be detrimental to flies and modified

Table 2.3: Mean pupal weight (mg) *G. austeni* by generations

Generations	Classes A	B	C	D	E
G0	12.9	15.6	19.9	22.0	25.3
F1	11.3	16.3	20.3	22.8	*
F2	14.0	16.8	20.5	23.4	*
F3	12.9	16.9	19.3	22.6	*
F4	13.1	16.5	21.0	22.2	24.0
F5	14.0	16.2	20.3	23.3	25.7
COLONY	12.9	16.9	20.4	23.5	26.4

* = very few or no pupae in the class

the insert design in cages, there was an indication that the pupal quality had improved with far less than 10% of pupae being in class A; the smallest group (Table 2.2). Similar positive indication was also observed for the pupal weights (Table 2.3). There was also an increase in pupal size through the generations.

After transfer of flies from the original TPU room, their performance in terms of pupal size and mean weight improved for all generations (data not shown). Note should be made that these flies were manually fed, further confirming that movement was detrimental to flies.

The overall productivity of flies on the TPU was unacceptable as more flies were going onto the TPU than pupae were being produced. It was clear that without major changes in cage construction and fly holding the TPU could not be developed into a practical unit for rearing tsetse. Nevertheless, the TPU did demonstrate that the principles of automated feeding and pupal collection are indeed valid. Experience gained from the TPU has lead to the development and design of a simplfied adult feeding and pupal collection system which is currently under evaluation with *G. pallidipes*. Considerable time was spent in 1997 on the design of this new system. Movement of cages will be limited to the time the cages are delivered for feeding and moved back to the pupal collection system.

2.2. NEW OPERATIONAL PROCEDURES

2.2.1. Self Stocking of Production Cages (SSPC)

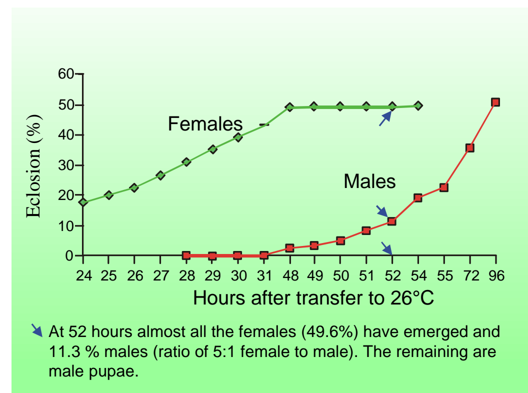
We demonstrated that the difference in the eclosion time of female and male tsetse may be exploited to develop a system whereby flies will emerge directly into production cages in the right number and sex ratio eliminating the need for chilling and manual separation of sexes, the so called “self stocking” of production cages. This could remove a very tedious step of collecting and sexing all newly emerged flies and will enable female flies to be easily removed from the males that are to be sterilized and released. In this system pupae are placed directly beneath the cage and flies emerge into the cage. The two systems initially tested included a light breaker circuit where flies were counted as they entered the cage and a weight system which recorded the reduction in weight of pupae following fly emergence. A third system has since been tested which uses the time elapsed from the initial emergence of a counted group of pupae to the point where the cage has been stocked by the right number of flies at the required sex ratio. This latter system was refined and demonstrated to be effective for *G. austeni.*

Pupae are collected on a daily basis, incubated in open trays at 23-24°C with 75% RH for 25 days. After this period a standard emergence cage is placed on the required number of pupae until the first fly emerges. (The number of pupae is calculated from the overall emergence rate and the female to male ratio required in the cage). At this point the pupae are moved to 26°C and placed under a production cage. The cages are removed after 24, 48, 72 and 96 hours and the number and sex of the flies in the cage is noted. It was observed that the critical period is between 48 and 72 hours so in the next series of experiments the cages were removed at hourly intervals 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. The production cage was required to be stocked with 100 females and 25 males. It was shown that for *G. austeni*, production cages can be stocked with the right number of flies in the required sex ratio of one male to four or five females, 52 hours from the onset of emergence (Fig 2.1). This self stocking system can result in tremendous savings in time and labour for a large facility.

Flies allowed to emerge using this system have been observed and pupal production is not different from cages that have been loaded manually. Pupal production/initial female (PPIF) at week 10 was 3.39 for SSPC cages and 3.67 for manually loaded cages. Pupae remaining after cages have been stocked are predominantly males and they could be irradiated and transplanted to the field site for release without having to be chilled and separated. The sexing error for males destined for release would be on average less than 0.5% females among males. Elimination of chilling and separation at emergence further reduces the time invested in handling young flies. Together with application of Day 0 mating and resident males (see Annual Report 1996), the total amount of time required for tsetse rearing has been reduced by 40%. Tests are currently underway with *G. pallidipes* to develop the same system.

Figure 2.1 Emergence pattern of *G. austeni* females and males at 26 °C



2.2.2. Chilled Adults Release System for Tsetse Flies

The chilled adult release system is a method of distributing sterile insects that has been used successfully for fruit fly, pink bollworm and screw worm SIT programmes. The adult flies are emerged, fed and reared to maturity in re-usable containers, immobilized by chilling in large refrigerated rooms and transferred to release machines in aircraft. They are then distributed in a free-fall manner along pre-determined swathes.

The release method that has been used for tsetse SIT progammes was initially by ground and subsequently by air using boxes which opened and released the flies. A predetermined numbers of chilled males which have been fed 2-3 blood meals prior to irradiation are placed in a bio-degradable cardboard box. The lid of the box is held in place by a rubber band and the boxes are loaded onto the aircraft. At the time of release the rubber bands are removed as the boxes are dropped through a chute on the floor of the aircraft along release swathes. For the proposed SIT component of the tsetse eradication programme in Ethiopia, a very large number of expensive boxes would be required. On grounds of cost and environmental concerns an alternative methodology is necessary.

To develop the chilled adult system the effect of prolonged chilling on the fitness of the fly needs to be assessed and preliminary tests were conducted using *G. austeni*. The main emphasis was on the biological requirements for males which would aid in the design of the release machine.

Male flies were given three blood meals and then divided into three groups. One group acted as control and the other two groups were chilled at 4-5°C for 24 or 48 hours respectively. At 24 and 48 hours, flies were allowed to recover at the insectary holding conditions, dead flies were removed and counted. The remaining flies were observed after one week for survival. In one test, surviving males were used for mating 1400 colony

Table 2.4: Mean [SD] survival of male *G. austeni* kept at 4-5°C for 24 and 48 hours and pupal production of females which mated with chilled males

Treatment	% survival	% survival at 1 week	PPIF	P/F/10d
24 Hours	97.83 [2.47]	85.33 [8.09]	4.34	0.72
Control 24 Hours	97.33 [2.08]	87.33 [6.66]		
48 Hours	90.33 [4.22]	54.33 [10.05]	4.2	0.65
Control 48 Hours	95.33 [3.06]	88.67 [5.13]		
Colony			4.71	0.89

PPIF = pupae per initial female
P/F/10d = pupae per female per 10 day

females and the pupal production during a 13 week period of these females was compared to that of a similar number of females mated by colony males.

Percent survival of flies was comparable for both control and the treated group kept for 24 hours at 4-5°C when assessed immediately after removal from low temperature and one week later (Table 2.4). After 48 hours temperature treatment, survival of chilled flies was lower than that of the control with 54.33 ± 10.05 % of chilled flies being alive after one week. The fecundity and therefore pupal production of females mated with such males was lower (PPIF = 4.2) than those of females mated by males which had been chilled for 24 hours (PPIF = 4.34). The colony figure used for fecundity (P/F/10d = 0.89) and pupal production (PPIF = 4.71) refer to females which were mated at the time of emergence at a ratio of one male to four females, the same as for the test flies except the males were one week old.

24 hour chilling appears not to affect the survival of flies, they recover instantly and as shown by production figures are able to inseminate females. Current tests are being conducted with *G. pallidipes* and the parameters being considered are the chilled holding/storage temperatures and the temperature used in the chilled drop machine in the aircraft. Apart from survival, mating competitiveness, insemination ability and recovery time will be investigated.

2.3. RE-ASSESSMENT OF FREEZE DRIED BLOOD

Vertebrate blood is the only dietary requirement for feeding tsetse but the supply of the blood is not always easy to guarantee. Originally tsetse were fed on live animals but with the development of the silicone membrane this changed. Warmed irradiated bovine blood collected from the slaughter house is presented through the silicone membrane. This is the system which was used to feed the large *G. austeni* colony at TTRI, Tanga from which males were used for the eradication programme on Zanzibar, Tanzania. The blood used in Tanga, amounting to about 20,000 liters was collected in Austria, processed and quality controlled in the Unit and then shipped frozen to the TTRI at Tanga. Shipping and storage costs of frozen blood are expensive undertakings.

In an effort to simplify the logistics of the provision of a quality blood diet for tsetse in future large scale SIT programmes on mainland Africa, the use of freeze dried blood was re-examined. Freeze dried blood had already been used on a small scale during an SIT tsetse programme in Nigeria. Work in the Unit looking at various aspects of the use of freeze dried blood for tsetse rearing had led to the conclusion that blood processed in this way could be reliably used as diet source for tsetse.

As well as freeze drying, the need for ATP, the need for pig blood and the effect of irradiation were assessed.

1. ATP, a phagostimulant found to improve blood intake among haematophagous insects, is an expensive additive to the diet and for that reason it was not used for the mass rearing *G. austeni* in Tanga.

2. Pig blood was found to be necessary for optimal pupal production for several tsetse species including *G. pallidipes*. However, in many African

countries, pig blood is not easy to obtain.

3. Freeze dried blood is normally irradiated after re-constitution and therefore requires a fairly large irradiation facility. If it can be irradiated as a dried product then this requirement is reduced.

The present study was designed to examine the above factors for their effect on *G. pallidipes*.

2.3.1. Effect of Radiation of Freeze Dried Blood on Survival and Productivity

Freeze dried blood was reconstituted either after or before irradiation or without any irradiation treatment. Fresh frozen blood from the batch from which the freeze dried blood was made, was used as control and the tests were carried out with both *G. pallidipes* and *G. austeni*.

2.3.1.1. *G. pallidipes*

240 fertilized *G. pallidipes* females were maintained on each of the following diets for seven weeks and survival and productivity were measured:

a) freeze dried blood, irradiated and then reconstituted (FDB/IR/RE)
b) freeze dried blood reconstituted and then irradiated (FDB/RE/IR)
c) freeze dried blood reconstituted and not irradiated (FDB/RE)
d) fresh frozen bovine blood, irradiated (FFB/IR)
e) fresh frozen bovine : porcine blood irradiated(75%: 25%), FFB/FFP/IR.

ATP was added to all the diets at a final concentration of 10^{-3} M and irradiation was at 1 kGy if required.

Flies maintained on freeze dried blood diets had lower mean percent survival compared to fresh frozen blood diets and the same was true of PPIF (Table 2.5). Survival however improved when irradiation took place after reconstitution. Flies maintained on freeze dried blood which was not irradiated survived better

Table 2.5: Survival and productivity of *G. pallidipes* maintained on different blood diets.

	FD/IR/RE	FD/RE/IR	FD/RE	FFB/IR	FFB/FFP/IR *
% survival	69.1	75.1	77.5	85.8	85.1
PPIF	2.11	2.46	2.29	2.51	2.56

* standard diet for *G. pallidipes* colony feeding

than those where the diet was irradiated either before or after reconstitution. The addition of porcine blood in the fresh frozen diet had no effect on either survival or production. These preliminary data support the previous results that freeze dried blood can be a good substitute for fresh frozen blood in *G. pallidipes*.

2.3.1.2. *G. austeni*

1400 fertilized *G. austeni* females were maintained on each of the diets mentioned above except for diet (e) for seven weeks.

Once again flies maintained on fresh frozen diets had better survival and pupal production. Irradiation after reconstitution appears superior to before reconstitution and the diet of reconstituted blood which was not irradiated had a better production (PPIF = 2.75) than irradiated freeze dried blood and approaching the performance of flies maintained on a normal diet of irradiated fresh frozen bovine blood, PPIF = 2.79 (Table 2.6).

These two preliminary tests showed that flies could be maintained on freeze dried

Table 2.6: Survival and productivity of *G. austeni* maintained on different blood diets

	FD/IR/RE	FD/RE/IR	FD/RE	FFB/IR
% survival	56.6	67.5	65.1	75.5
PPIF	2.24	2.64	2.75	2.79

blood and that they would produce the number of pupae required for a self

Table 2.7: Mean survival [SD] (%) and quality factor (QF) of *G. pallidipes* maintained on different diets

ATP Concentration	% survival [SD] FF	% survival [SD] FD	Quality factor [SD] FF	Quality factor [SD] FD
10^{-3} M	94.33[5.13]	96.67[3.50]	1.13[0.12]	1.16[0.12]
10^{-7} M	95.33[4.04]	92.00[1.73]	1.25[0.03]	1.17[0.06]
0	94.67[2.30]	94.33[2.30]	1.18[0.01]	1.16[1.16]

sustaining colony which is, PPIF = 2.1 in seven weeks. The performance of flies for all the diets were above PPIF of 2.1.

2.3.2. Effect of ATP and Freeze Dried Blood on *G. pallidipes*

Ninety fertilized females (3 cages each with 30 females) were maintained for 25 days on diets of fresh frozen or freeze dried blood supplemented with various concentrations of ATP. Survival and a standard quality factor were calculated and all diets were irradiated with 1 kGy before use. Pupae were classified according to size.

The survival and quality factor obtained with diets containing various concentrations of ATP showed that removal of ATP from the diet did not have major impact on tsetse survival (Table 2.7) The standard quality factor which measures the quality of blood which enables a female fly to live and produce a pupa during the first 25 days of life was also acceptable for all diets(QF minimum value = 1).

Pupal quality as measured by size showed that the distribution for all the groups was similar with the exception of diets without ATP which had a large proportion of class E pupae, the largest (Table 2.8). This value was 12.7% for fresh frozen blood and 5.09% for freeze dried blood. However, the difference was not statistically different by the Tukey HSD test. Experiments are planned to determine the effect of long term maintenance of *G. pallidipes* on freeze dried blood diets without ATP and the role of pig blood .

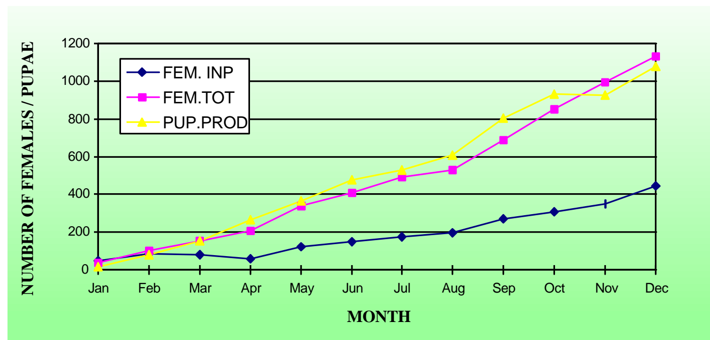
Table 2.8: Pupal size classes (%) of pupae produced by *G. pallidipes* maintained on different diets

ATP Conc. Classes	Fresh Frozen 10^{-3}M	Fresh Frozen 10^{-7}M	Fresh Frozen 0	Freeze dried 10^{-3}M	Freeze dried 10^{-7}M	Freeze dried 0
A	1.70	0.00	0.00	0.00	1.58	1.69
B	11.86	10.00	11.11	20.96	11.11	13.56
C	59.32	47.14	52.38	56.45	57.14	59.32
D	23.72	41.42	23.81	20.96	28.59	20.34
E	3.40	1.44	12.70	1.63	1.58	5.09

2.4. *G. PALLIDIPES* COLONY FROM ARBA MINCH, ETHIOPIA

In an effort to establish a colony of *G. pallidipes* from the Southern Rift Valley, Ethiopia for the proposed tsetse eradication project, a field insectary was established at Arba Minch to house field caught flies. The field caught females were maintained on live hosts and the pupae produced were shipped to Seibersdorf to initiate a colony. Pupal shipments began in August 1996 and continued to early July 1997. During this period 9334 pupae were received in Vienna from which 2263 females and 2060 males emerged. The overall emergence rate was 46.36%. Survival of flies that emerged was disappointing with many flies dying within 24 hours. Coupled with the low emergence rate, only 631 females survived to start the colony. Dissections of unemerged pupae showed that 67.9% of the pupae were lysed i.e. died before any development took place, 21.7% died at an early stage without melanization and 9.5% were fully pigmented but with wet cuticle and 0.9% were fully formed and ready to emerge. These observations pointed to poor handling or shipment of very young pupae although even hand carried pupae had similar low emergence rates. Flies that survived fed readily on membranes and were kept separate by generation. By the end of December 1997 there were 4 generations with a total of 1129 females (Fig. 2.2).

Figure 2.2. Development of G. pallidipes originating from Arba Minch, 1997



The mean pupal weight for all the generations was good (Table 2.9) and encouraged the pooling together of flies from generation 5 onwards.

Large scale field collection of flies is being organised for 1998 to enable for a more rapid build up of the colony to take place.

2.5. OTHER TOPICS

2.5.1. Improvements on Routine Colony Maintenance

Having demonstrated with *G. austeni* that there was no need to mature the sexes separately before mating and that when fewer males are put in the production cages together with females they could be left together without the need for separation without compromising performance, colony maintenance for the other species of flies have been revised accordingly. All species are mated at emergence (Day 0 mating with resident males) and ratios at mating are one male to four females for *G. morsitans*, *G. fuscipes* and *G. palpalis* and one male to three females for *G. pallidipes* and *G. brevipalpis*. The *G. pallidipes* strain from Arba Minch is still kept on the previous regime where sexes are kept separate at emergence, mating takes place between 7 day old females with males 10+ days old in a sex ratio of 1:1 ratio for 3 days before chill separation. Chilling for separation of sexes is now only carried out at the time of emergence when production cages are loaded with flies. No immediate adverse effect of these changes has been observed.

Table 2.9: Mean pupal weight [SD] in mg for *G. pallidipes* colony originating from Arba Minch, Ethiopia

Generation	Mean pupal weight	Period of observation
G0	33.87[4.64]	Dec. 96 - Jul. 97
F1	34.82[4.29]	Jan. 97 - Nov. 97
F2	35.27[4.18]	Mar. 97 - Dec. 97
F3	35.29[4.38]	Jun. 97 - Dec. 97
F4	34.93[3.18]	Sep. 97 - Dec. 97

Because of the cost of ATP as an additive to the diet and due to the fact that it was not used for the colony of *G. austeni* at Tanga TTRI, it was felt necessary to review the need for its use in the diet. The revealed confirmed that it is not necessary and it will be removed from the diet of all species during 1998.

During 1997, there was a reduction in the colony size of *G. austeni* colony since the flies were no longer required as a back up for the eradication project in Zanzibar. The *G. pallidipes* Uganda strain has been increased in size to provide material for experiments and for evaluation of the new automation procedures pending the build up of the Arba Minch *G. pallidipes* colony.

2.5.2. Population Genetics

The need for sensitive PCR based tools to analyse population variation in tsetse will become essential to interpret patterns of fly movement and distribution in connection with the tsetse SIT component of the eradication project in Ethiopia. The tools are necessary to elucidate migration of flies in order that effective barrier systems can be devised to protect fly free areas. Single strand conformation polymorphism (SSCP) is a new, very sensitive technique which can visualize base-pair changes in DNA. A consultant, Dr. E. Krafsur, Iowa University introduced this technique into the Unit and

demonstrated its use with *G. pallidipes*. Using PCR primers, portions of the mitochondrial genome were amplified and then heated to 95°C to denature the DNA into single strands. This product was then run on a polyacrylamide gel and the bands visualized using silver staining. Genic diversity was readily observed at several mitochondrial loci which has since been confirmed in subsequent experiments. The technique, whilst being extremely sensitive is laborious and will require extra support if it is to be carried out on a large scale

2.5.3. Infection of *G. pallidipes* with Wolbachia Spps

Wolbachia are symbiotic bacteria that when present in the reproductive organs of insects can cause cytoplasmic incompatibility, a form of naturally occuring sterility. Tsetse species differ in whether or not they are infected with this bacteria. *G. pallidipes* appear not to be infected whereas *G. austeni, G. brevipalpis,* and *G. morsitans* are. Dr. Bourtzis from Yale attempted to transfer *Wolbachia* from infected Glossina species to *G. pallidipes*. The attempted infection was carried out by injecting cytoplasm from eggs from infected individuals into the haemocoele of non-infected females. The presence and absence of the bacteria was monitored using specific PCR primers. No clear evidence of cross species infections were obtained but it was shown that the transfer of certain types of cytoplasm between the different tsetse species caused high levels of mortality in the recipients.

Table 2.10: Tsetse pupae supplies distributed to other centres in 1997

Glossina austeni		
Dr. H Herren	51,000	ICIPE, Nairobi, Kenya
Dr. D Moloo	46,000	ILRI, Nairobi, Kenya
Dr. Maudlin	4,600	University of Glasgow, UK
Dr. Gariou-Papalexiou	2,800	University of Patras, Greece
TC Project	2,000	TIRI, Tanga, Tanzania
Dr. K Bourtzis	400	Yale University, New Haven, USA
Dr. Bennettova	200	Academy of Science, CR
Dr. G Liebisch	100	Vet. Univ., Hannover, Germany
Prof. H Wetzel	100	Vet. Univ., Hannover, Germany
Dr. Zacharopoulou	100	University of Patras, Greece
Dr. Service	100	Tropical Medicine, Liverpool, UK
Sub Total	**107,400**	
Glossina palpalis		
Dr. Maudlin	11,560	University of Glasgow, UK
Prof. Molyneux	10,175	Univ. of Salford, UK
Dr. S Aksoy	2350	Yale University, New Haven, USA
Dr. L Auerswald	300	Univ. Cape Town, SA
Dr. Bennettova	300	Academy of Science, CR
Dr. G Liebisch	150	Vet. Univ., Hannover, Germany
Prof. H Wetzel	130	Vet. Univ., Hannover, Germany
Sub Total	**24,965**	
Glossina brevipalpis		
Dr. Guerin	4,770	Univ. Neuchatel Switzerland
Dr. D Moloo	1,600	ILRI, Nairobi, Kenya
Dr. Bennettova	550	Academy of Science, CR
Dr. K Bourtzis	450	Yale University, New Haven, USA
Prof. H Wetzel	100	Vet. Univ., Hannover, Germany
Sub Total	**7,470**	
Glossina fuscipes		
Dr. Melaku-Girma	7,000	ICIPE, Nairobi, Kenya
Dr. S Akinyi	1,550	ICIPE, Nairobi, Kenya
Prof. R Disko	300	University of Munich, Germany
Sub Total	**8,850**	
Glossina pallidipes		
Dr. K Voskamp	100	Animal Physiology, Haren, Netherlands
Dr. Bennettova	100	Academy of Science, CR
Sub Total	**200**	
Glossina morsitans		
Dr. Maudlin	30,410	University of Glasgow, UK
Prof. A Tait	4,850	University of Glasgow, UK
Dr. S Aksoy	2,720	Yale School of Medicine, New Haven, USA
Dr. J. Zdarek	1,420	Academy of Science, CR
Dr. R Brun	1,300	Tropical Institute, Basel, Switzerland
Dr. G Zollner	550	National Resources Inst., Chatham, UK
Dr. Tiedjen	300	Tropical Medicine, Berlin, Germany
Dr. Turner	100	University of Glasgow, UK
Sub Total	**41,650**	

2.5.4. Supply of Material to Counterparts.

Table 2.10 gives a summary of the flies and blood provided to counterparts in 1997. Many of the recipients rely totally on the supply of material from Seibersdorf for their research. In addition to the material shown in the table a total of 12,000 L of fresh frozen quality controlled blood was supplied to the TTRI facility in Tanga.

3. MEDFLY GROUP

The present focus of this group is the development, testing and transfer of genetic sexing strains (GSS) including technical support for programmes which utilize these strains. The medfly remains of key agricultural importance mainly because of its significance as a quarantine pest. The need to establish fly free areas for this pest has focused more and more attention on the use of the SIT with the associated demand for a more efficient approach. GSS can partially fulfil this demand by allowing the release of predominantly males. The whole process from development to implementation is carried out by the Unit. This section of the report is presented in the order with which the project has evolved, namely, development 3.1, mass-rearing 3.2 and field cage testing 3.3.

3.1. GENETIC SEXING

As indicated in the Introduction, GSS in medfly are now being used in operational medfly SIT programmes in several countries. The experiences gained during the use of these strains has suggested ways that they can be improved. The work reported in this section is focussed on the improvement of the existing GSS and the development of new strains based on the requirements of the end user and on the accumulated experience. The technology has to be made cheaper, more robust and more applicable for other pest insect species by developing transgenic techniques. Furthermore, additional features can be added using genetic or molecular techniques that are not available in the current strains, e.g. unique, inheritable tags for the secure identification of released flies.

3.1.1. Long Term Evaluation of GSS

Several GSS have been reared for up 63 generations at a level of approximately 1500 flies per generation to observe stability. Each generation, 40 ml of pupae were sampled and the resulting adult flies

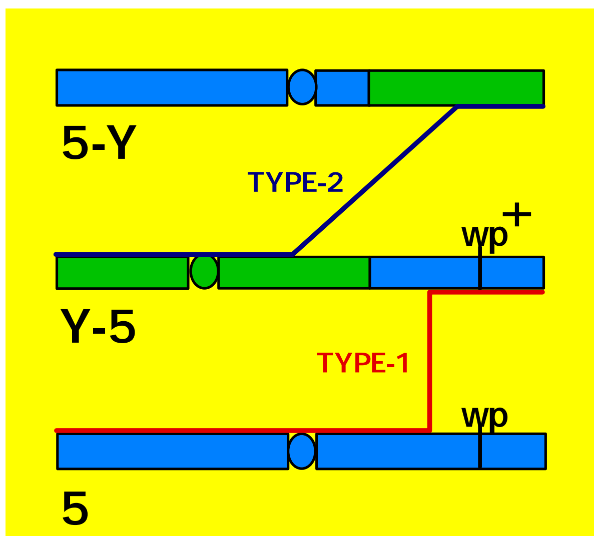
were counted and sexed. A parallel sample of pupae (34 ml) was used to set up the following generation without the removal of any recombinant individuals. This standard procedure was used to determine the long term stability of GSS, in addition to the evaluation of certain quality control (QC) parameters of particular relevance to GSS.

3.1.1.1. Stability

In GSS, males are phenotypically wild-type and females are mutant for the selectable gene(s) based on which the sexes can be separated. Due to the construction of GSS using reciprocal Y-autosome translocations, two types of genetic recombination can occur in the males carrying the translocation and this can lead to the deterioration of the sexing system (Figure 3.1).

Type-1 genetic recombination takes place between the part of the autosome which carries the wild-type gene and is translocated to the Y chromosome and the free autosome which carries the mutant gene. Recombination occurs in the region between the translocation breakpoint and the gene. This results in the production of wild-type females which have a selective advantage over the homozygous mutant females and depending on the rearing conditions, recombinant females begin to accumulate in the colony. These females cannot be separated from the males and therefore they reduce the accuracy of the sex separation and contaminate male production. The recombination frequency is dependent on the structure of the Y-autosome translocation, i.e. on the distance between translocation breakpoint and the selectable gene(s). In the initial medfly GSS this distance was relatively large (e.g. T(Y;5)30C, Figure 3.2) and this led to the rapid deterioration of the sexing characteristics of the strains, often within 8 generations. This prohibited their use in large scale SIT programmes.

Figure 3.1 Chromosome structure of GSS males and two possible types of recombination. The autosome (blue), linked to the male determining Y chromosome (green), carries the wild-type gene (*wp*+) and the free autosome carries the mutant gene (*wp*). Genetic recombination in males can be type-1 (autosomal) or type-2 (intra-Y-chromosomal)

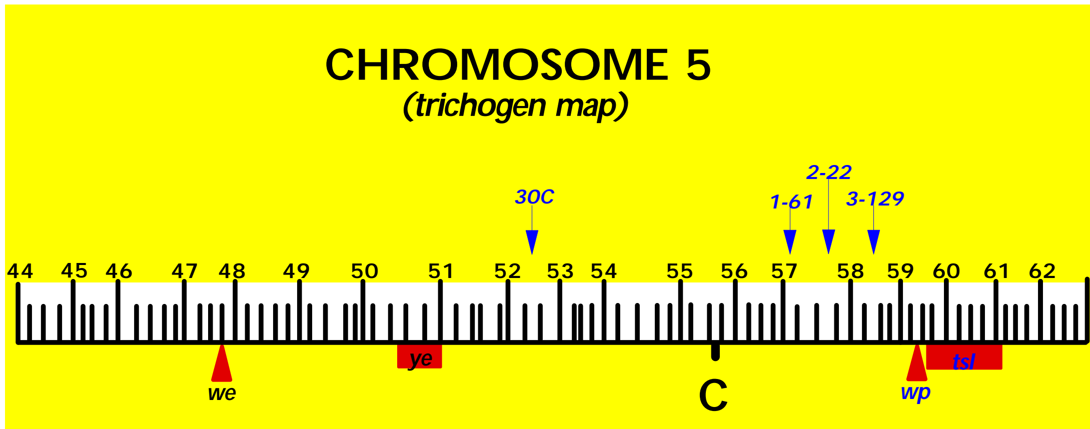


Type-2 genetic recombination takes place between the two translocated Y-chromosomal fragments. The result is a free Y chromosome that is shorter than the normal Y chromosome, in extreme cases a very small chromosome is produced. Due to the fact that males carrying this free Y chromosome are fully fertile, they accumulate rapidly in the colony, they are also mutant. Type-2 recombinant males do not affect the accuracy of the sexing procedure but they do reduce the productivity of the mass rearing.

In Table 3.1 the results of long term rearing of several GSS are summarized. The strains differ in two respects, the selectable gene(s) and the translocation.

Two different genes have been investigated, white pupae (*wp*) which allows optical sex separation of the sexes at the pupal stage and a temperature sensitive lethal (*tsl*) which allows elimination of females at the egg stage using a high temperature treatment. For practical reasons, all *tsl* strains also contain *wp* as an additional marker as a way to monitor strain stability. In other GSS that were not intended for use in field

Figure 3.2: Schematic representation of the medfly chromosome 5. The map positions of four genes are shown in red. Two of them (white eye, *we* and yellow body, *ye*) are only used for the genetic analysis, while the other two (white pupae, *wp* and temperature sensitive lethal, *tsl*) are used as selectable genes in GSS. The breakpoints of four Y-autosome translocations used in GSS are indicated by blue arrows. C=centromere.



programmes additional genes (white eye, *we*; and yellow body, *ye*) were included to allow for a better genetic analysis.

Three new Y-autosome translocations were generated in which the breakpoints are unlike GSS 30C, close to the selectable gene(s) (Figure 3.2). VIENNA 4 carries translocation T(Y;5)1-61 and VIENNA 6 carries translocation T(Y;5)2-22. The additional letters in the description refer to independent reconstructions of this strain from the principle components, i.e. from the *wp tsl* strain and the translocation strain. Both of these GSS have been used in several mass rearing facilities with pupal production levels of up to 300 million per week. VIENNA 7 is a new strain that has not yet been transferred to an operational facility and it carries translocation T(Y;5)3-129. SEIB 6-95 and SEIB 6-96 are two strains based only on the *wp* gene and were maintained as standard strains for mass rearing projects in Crete and Argentina, they both carry T(Y:5)2-22.

Table 3.1 shows that long term stability is, under the conditions used here, very good. Strains can be reared for up to 63 generations without losing their sexing

Table 3.1: Long term stability of different medfly GSS.

Strain	Generations	wp^+ Females	wp^+ Males	*wp* Females	*wp* Males	% Recombinants
VIENNA 4 A+B	26	2	23332	14448	12	0.037
4C	63	27	61331	47940	1	0.026
4E	42	5	40356	30386	6	0.016
total	**131**	**34**	**125019**	**92774**	**19**	**0.024**
VIENNA 6	9	1	6897	4657	0	0.009
6B	37	15	39576	28840	2	0.025
6C	37	3	35745	24815	5	0.013
6E	10	1	10837	6102	2	0.018
6HE	36	15	37794	25667	5	0.032
total	**129**	**35**	**130849**	**90081**	**14**	**0.023**
VIENNA 7C	6	0	5685	4527	0	0.000
7D	25	9	24861	19164	6	0.034
7E	13	5	13546	10608	0	0.021
total	**44**	**14**	**44092**	**34299**	**6**	**0.026**
SEIB 6-95	36	6	39014	26010	6	0.018
-96	24	4	27059	18054	2	0.013
total	**60**	**10**	**66073**	**44064**	**8**	**0.016**
Grand total		**93**	**366033**	**261218**	**47**	

characteristics. The recombination frequencies ranged from 0.023% to 0.026% in strains based on *wp tsl*, i.e. they are very low and virtually identical in all strains. It can be seen that the two reciprocal types of recombinants, wild type (wp^+) females and mutant (*wp*) males, do not occur with equal frequency, i.e. more recombinant females were detected. This may be due to the fact that in these strains the recombinant males are also mutant for *tsl* which could result in a reduced viability. In the two *wp*-based strains the recombination frequency is slightly lower, 0.016%, and both types of recombinants occur with equal frequency. Most importantly, in none of the cases studied here, was any accumulation of type-1 or type-2 recombinants observed. Type-2 recombination seems to occur so infrequently that it was never observed in this size of small scale rearing. From the numbers presented here it can be estimated that the maximum frequency for type-2 recombination is 1.6×10^{-6}.

3.1.1.2. Genetic behavior of Y-autosome translocations

All males in GSS carry a Y-autosome translocation linking the wild-type gene to the male sex. Such chromosome rearrangements cause males to be only 50% fertile due to 50% of sperm being genetically balanced (alternate segregation) whilst 25% carry a deletion and 25% carry a triplication (adjacent-1 segregation) and are genetically unbalanced (Figure 3.3). In the GSS so far studied alternate and adjacent-1 segregation occur with equal frequency. Eggs fertilized by genetically unbalanced sperm are not completely viable with a deletion causing lethality during the embryo stage and a triplication, being less damaging, causing lethality in the larval, pupal or even adult stage.

One of the QC parameters that is affected by the survival of triplication males is fly emergence. A large proportion of triplication males are unable to emerge completely from the pupal case and are classed as half-emerged. Table 3.2 summarizes these data for several different GSS. As an internal control the percentage of half emerged females is used and it ranged from 3% to 7% depending on the gene used. In the males of VIENNA 4 and VIENNA 6 much higher values are observed (11.4% and 20%, respectively) and in SEIB 4 and SEIB 6, the values are even higher (18.6% and 26.7%, respectively). These very high frequencies of half emerged males are of great concern to managers involved in mass rearing and field releases even though they have little impact on the programme.

For VIENNA 6, SEIB 6 and SEIB 4 it was shown that the high level of half emerged males was caused solely by the survival of the triplication individuals (Table 3.2) To

Figure 3.3: Segregation of a Y-autosome translocation during meiosis. Alternate segregation produces balanced offspring leading to an overall male fertility of 50%. Adjacent-1 segregation produces two types of genetically unbalanced sperm; half of them carry an autosomal deletion and the other half carry an autosomal triplication. In order to monitor the survival of unbalanced individuals a second gene *we* is included in the strain.

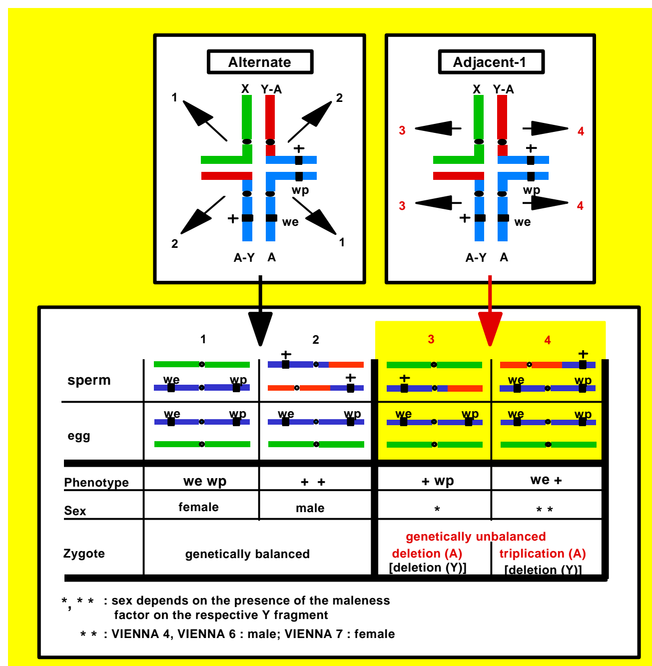


Table 3.2: Survival of triplication carrying individuals in medfly GSS. (ND = these measurements cannot be obtained)

Strain	Marker [s]	% Half Emerged Females	% Half Emerged of All Males	% Balanced of All Males (% Half Emerged)	% Triplication of All Males (% Half Emerged)
VIENNA 4A/B	*wp/tsl*	8.3	11.7	ND	ND
4C	*wp/tsl*	5.2	9.4	ND	ND
4E	*wp/tsl*	6.1	13.2	ND	ND
average		6.53	11.43		
VIENNA 6C	*ye/wp/tsl*	6.8	17.7	87.9 (7.5)	12.1 (91.2)
6E	*ye/wp/tsl*	3.8	19.7	86.3 (8.3)	13.7 (91.3)
average		5.3	18.7	87.1 (7.9)	12.9 (91.2)
VIENNA 6A	*wp/tsl*	3.1	15.2	ND	ND
6B	*wp/tsl*	6.4	23.8	ND	ND
6HE	*wp/tsl*	6.7	20.9	ND	ND
average		5.4	19.97		
VIENNA 7C	*ye/wp/tsl*	5.8	4.1	100	0.00 (female)
7D	*ye/wp/tsl*	6.7	4.1	99.97	0.03 (female)
7E	*wp/tsl*	4.5	2.2	ND	ND
average		5.67	3.47		
SEIB 4	*we/wp*	1.3	22.8	72.2 (1.3)	27.8 (78.8)
42	*wp*	4.6	14.4	ND	ND
average		2.95	18.6		
SEIB 6	*we/wp*	1.3	27.7	65.8 (3.2)	34.2 (74.7)
6-95	*wp*	3.6	27.6	ND	ND
6-96	*wp*	4.0	24.8	ND	ND
average		2.97	26.7		

recognize these genetically unbalanced flies an additional gene e.g. *ye* or *we*, both located on the left arm of chromosome 5, has to be included in the strain. Here the balanced males are wild-type while the triplication carrying males are mutant for the additional gene (eg *we* wp^{+} in Figure 3.3). Table 3.2 shows that the percentage of half emerged balanced males in these strains is almost the same as in the females whereas between 75% and 91% of the triplication carrying males were half emerged. As mentioned earlier, a Y-autosome translocation causes 50% total sterility but this effect is not always expressed at the egg stage because of the survival of triplication individuals. The viability of the balanced males is completely normal in every respect. In GSS where balanced and triplication carrying flies cannot be distinguished, the percentage of half emerged flies is high leading to a low QC value.

With translocation 3-129, (VIENNA 7), the triplication type offspring are female and emerge from wild type pupae. In addition, these females are much less viable with a large proportion dying before reaching the pupal stage and only very few adults emerge. As a consequence, the percentage of half emerged males (3.5%) and females (5.7%) is not very different from the control. Table 3.3 summarizes these results and compares the three different translocations combined with either *wp* or *wp tsl* as selectable genes. Using the percentage of half emerged flies as an indicator for the frequency of triplication carrying offspring, the strains can be described as follows:

a) If only *wp* is used as a selectable gene, more triplication carrying flies appear as half emerged individuals than if *wp* and *tsl* are used. Apparently, the presence of two mutations, especially a temperature sensitive lethal, reduces the viability of the unbalanced

Table 3.3: Percentage of half emerged flies for three different translocations.

Selectable Gene(s)	Translocation 1-61		Translocation 2-22		Translocation 3-129	
	Female	Male	Female	Male	Female	Male
wp	3	19	3	27		
wp tsl	7	11	5	19	6	4

individuals and they die before the end of the pupal stage

b) In GSS with translocation 2-22, more triplication carrying individuals are detected at this late stage than with translocation 1-61. The reason is that the triplication in 2-22 is shorter than in 1-61.

c) In GSS with translocation 3-129 in combination with *wp tsl,* very few triplication individuals appear at the pupal/adult junction with the consequence that the QC parameter "half emerged males" is as low as in a normal, bisexual strain. Furthermore, a large proportion of the unbalanced individuals die before reaching the pupal stage. This is very different from the other two types of GSS and allows for a more cost effective rearing. Furthermore, it avoids the risk that batches of pupae are sent to the field containing a high proportion of ineffective triplication carrying individuals.

Table 3.4: Survival of 1000 eggs in two different *tsl* based GSS at two temperatures.

Stage	25°C VIENNA 6	25°C VIENNA 7	34°C VIENNA 6	34°C VIENNA 7
Larvae	773	823	490	491
White Pupae	216	216	0	0
Brown Pupae	472	343	362	272
Females	209	210	0	0
Balanced Males	247	250	211	230

The above discussion refers primarily to the rearing of the GSS at 25°C. In strains where the *tsl* is used as a selectable gene the occurrence of triplication carrying individuals is less of a problem as they are, at least to a certain degree, temperature sensitive and die during the temperature treatment. Table 3.4 shows a comparison of the strains VIENNA 6 and VIENNA 7. At 25°C, only the embryos carrying deletions die and, as a consequence, reduce the egg hatch by 25%. At 34°C, egg hatch is further reduced to approximately 50%, a value expected if all balanced females die in addition to the deletion carrying embryos. At both temperatures 25% fewer brown, male pupae are recovered in VIENNA 7. However, this is beneficial as the brown pupae recovered in VIENNA 6 in excess of 250 per 1000 eggs were shown to be unwanted triplication carrying individuals. This becomes very obvious if the number of male adults is compared where both strains produce nearly equal numbers of balanced males and both are very close to the theoretical value of 250 per 1000 eggs. For VIENNA 6 the emergence from brown pupae is 52% at 25°C and 58% at 34°C, in case of VIENNA 7 the respective values are much better, i.e. 73% and 85%, very close to a normal bisexual strain.

VIENNA 7 is currently under investigation by the medfly rearing group (see section 3.2.2) to verify its improved characteristics and to determine whether type-2 recombination occurs in this strain. This recombination is so rare that it can only be observed during mass rearing. In VIENNA 7 the breakpoint on the Y chromosome is close to the centromere and the expectation is that recombination between the two translocated Y fragments should be extremely rare.

3.1.2. Transformation of Medfly Embryos

One of the key requirements for the molecular manipulation of pest insects is a functional transformation system. The required components are described in the Annual Report of 1996. In collaboration with a consultant, Al Handler (Gainesville), a large transformation experiment was performed to test the transposable element *piggyBac* for its suitability as a transformation vector for the medfly.

3.1.2.1. Microinjection of medfly embryos

Four parallel experiments were set up with different helper plasmids and different vector constructs:

Experiment 1:
helper: pBΔSst (*piggyBac* transposase with original promoter),
vector: pB[Cw]-6 (*piggyBac* with medfly white gene controlled by a heatshock (hsp70) promoter)

Experiment 2:
helper: hsp70-pBΔSst (piggyBac transposase with heatshock (hsp70) promoter,
vector: as in experiment 1

Experiment 3:
helper: pBΔSst, vector: pB[Cw/PUb-nls-EGFP] (as in experiment 1 plus enhanced Green Fluorescent Protein Gene (Clontech) with polyubiquitin promoter and nuclear localization signal)

Experiment 4:
helper: pBΔSst,
vector: pB[Cw/hsp-tra-lacZ] (as in experiment 1 plus a transformer gene (Drosophila) - lacZ construct with hsp promoter)

In total, 5680 embryos were injected (Table 3.5). As expected, the recovery of larvae is reduced significantly but compared to a previous experiment (32.8%, Annual Report 1996) the recovery is better. After the injection, surviving larvae were transferred to standard larval medium. The larval and pupal rearing temperature was reduced from 25°C to 20°C to slow down development. Table 3.5 shows that pupal and adult recoveries were nearly unaffected by the microinjection of the embryos. The overall recovery was significantly higher than in the previous experiment, 33% versus 13%.

Table 3.5: Survival of medfly embryos injected with various transformation constructs.

Experiment	Embryos Injected	Larvae (%)	Pupae (%)	Flies (%)	% Survival Total
1	1800	692 (38.4)	477(68.9)	432 (90.6)	24.00
2	1500	713 (47.5)	565 (79.2)	455 (80.5)	30.33
3	1180	718 (60.9)	638 (88.9)	556 (87.2)	47.12
4	1200	705 (58.7)	609 (86.4)	415 (68.1)	34.58
Total	5680	2828 (49.8)	2289 (80.9)	1858 (81.2)	32.71

3.1.2.2. Test crosses with the surviving adults

The surviving flies (G0) were used to set up crosses as shown in Table 3.6. In addition, 65 single G0 female and 50 single G0 male crosses were set up for experiment 2. [Among the G0 flies, one was discovered where parts of the eyes were wild-type while others were white. However, crossing this individual with *we* gave only mutant offspring and, consequently, it has to be assumed that the partial rescue of the eye color phenotype was caused by transient expression of the we^{+} gene of the vector without inheritable integration into the genome]. Eggs were collected every second day for 16 days. To extend the screening of the G1 adults, larval and pupal development was slowed down by incubation at 20°C. Starting four days after egg collection all samples were treated every day for one hour with 37°C to induce activity of the heatshock promoter. This treatment was continued until the first flies emerged.

The G1 generation was screened for the presence of flies with complete or partial rescue of the mutant white eye color, the potential transformants are expected to show at least some degree of red pigmentation. Only in experiment 2 were two potential transformants detected (0.022%). In the other experiments no flies with detectable levels of red pigmentation were found despite the fact that in all experiments very large numbers were screened (experiments 1, 3 and 4 combined: 32142 G1 flies; experiment 2 (mass and single pair crosses): 20265 G1 flies). In the single G0 crosses of experiment 2, two families were

discovered where some flies show a more or less wild type eye color. One family was found out of 38 successfully mated females and one among 25 successfully mated males, 2.6% and 4%, respectively. It is difficult to explain the absence of other transformants, especially in experiment 1, as the same helper/vector combination was used successfully by Al Handler at the University Hawaii in an earlier experiment.

During this screening, several mutant phenotypes were observed (Table 3.6) that did not affect the eye colour but in all cases the head or its bristles were affected. One of the mutant phenotypes resembled a known mutation, double chaetae (dc, chromosome 4). All flies with dc bristles had also a rough eye surface. It is not known yet whether also the original dc has this second effect. The latter phenotype is very difficult to see and may have been overlooked in the past. The frequency of mutant individuals averaged 0.036% in experiment 1, 3 and 4 and was significantly higher in experiment 2: 0.252%. Also the single pair crosses in experiment 2 showed a very high level of mutant phenotypes (0.341%). It appears that this elevated frequency of mutations coincides with the occurrence of transformants, i.e. in experiments with a high mutation frequency the probability to find transformants is also high.

3.1.2.3. Crosses with mutant flies and potential transformants

The potential transformants (G1) were crossed individually with *we* flies. As described above, a heat shock treatment was applied. Nine families produced offspring and we are currently analyzing the G2. Preliminary results show that in

Table 3.6: Mutant phenotypes produced following the injection of medfly embryos with different transformation constructs and mating the resulting flies with *we*

Experiment	Cross	Flies Screened	Exceptional Flies Recovered
1	100 *we* males crossed with 165 G0 females	9571	mutant phenotypes : 1 female deformed head
	165 G0 males crossed with 300 *we* females	8489	mutant phenotypes : 3 males rough eyes, *dc* bristles 3 females rough eyes, *dc* bristles
total: (%)		**18060**	**7**
2	100 *we* males crossed with 165 G0 females	2177	mutant phenotypes : 2 males rough eyes, dc bristles 6 females rough eyes, dc bristles 1 male without SOF bristles 1 male deformed head
	165 G0 males crossed with 300 *we* females	6933	mutant phenotypes : 5 males rough eyes, *dc* bristles 1 female rough eyes, *dc* bristles 3 males without SOF brisles 4 female deformed head **potential transformants : 1 male light red eyes 1 female red eyes**
total: (%)		**9110**	**13** **potential transformants: 2**
3	100 *we* males crossed with 200 G0 females	5629	mutant phenotypes : 1 male rough eyes, *dc* bristles
	165 G0 males crossed with 300 *we* females	2561	mutant phenotypes : 2 females rough eyes, *dc* bristles
total: (%)		**8190**	**3**
4	100 *we* males crossed with 200 G0 females	4526	mutant phenotypes : 1 female deformed head
	100 *we* males crossed with 200 G0 females	1366	mutant phenotypes : 1 male deformed head
total: (%)		**5892**	**2**

all families the wild-type eye phenotype is inherited correctly, i.e. as a dominant trait. The intensity of the eye color is somewhat variable even among the offspring of a single parent. Furthermore, the eye color never reaches the same dark red/brown color as found in the wildtype. The appropriate crosses have been set up to generate lines that are homozygous for a single insertion. Molecular tests on these lines will be required to verify the presence of the transgene. Furthermore, it is planned to map the insertion site cytologically by *in-situ* hybridization to polytene chromosome.

The mutant flies discovered in the screen of the G1 generation were pooled according to their phenotype and were inbred, however without heat shock treatment. Currently, we have started to analyze the G2 flies. The *dc* phenotype is apparently also inherited as a normal dominant trait but analysis is complicated by the fact that *dc*, as the original mutation, shows significant variability of penetrance. The eye color of these flies is generally white and only in a few cases does the color tends towards yellow which could be an indication that the white gene is expressed although at very low levels. The parents also showed no obvious pigmentation of the eyes despite the fact that they were heat shocked. Further analysis is required to understand the nature of these new mutations but it could represent a way to clone the respective genes.

3.1.3. Other Areas of Ongoing Research

3.1.3.1. Screen for pericentric inversions

Pericentric inversions are valuable genetic tools to suppress type-1 recombination. They could be very useful for the construction of GSS (e.g. to facilitate the outcrossing with wild flies), for the stability of GSS (i.e. no type-1 recombination in the heterozygous males), and as a general genetic tool (i.e. as a balancer chromosome). After the first successful screen reported in the Annual Report 1996, a second one was initiated. In total, 1400 single pair crosses were set up. The analysis of candidate families through genetics and cytology (in collaboration with Antigone Zachararopoulou, Patras) is ongoing.

3.1.3.2. Analysis of type-2 recombinants and the segregation of deleted Y chromosomes

Type-2 recombinants were isolated from different mass rearing colonies, including the large colony in Mendoza, Argentina. They were analyzed genetically and cytologically (in collaboration with a consultant, Antigone Zacharopoulou, Patras). It was shown that they carry free, but partially deleted Y chromosomes and that they are fully fertile. Two Y chromosomes with very long deletions were investigated in view of generating new translocations for GSS (without type-2 recombination) and as starting material for the isolation of the maleness factor. In the course of these preliminary experiments it was discovered that males carrying very short Y chromosomes show a phenomenon reminiscent of the X chromosome loss in *Drosophila* resulting in gynandromorphs. Experiments are ongoing to investigate this phenomenon further.

3.1.3.3. Determination of mtDNA haplotype in different strains and populations

Investigation of the mitochondrial DNA (mtDNA) haplotype was initially aimed at discriminating between three particular strains. Subsequently, this analysis was expanded as material from different wild populations became available. In the course of these experiments, strains were discovered where the mtDNA has a very unique restriction pattern that makes them distinguishable from all other strains/populations tested so far. This can be exploited by generating new GSS in such a way that this particular mtDNA haplotype is introduced through the maternal lineage. The resulting strain carries an internal marker that can be used to clearly identify this strain and to monitor its integrity. Preliminary small scale tests have also shown that mtDNA

remains relatively stable in dead flies and can be analyzed in material that is close to two weeks old. This may open the possibility to use the mtDNA marker also on trapped flies from the field.

3.1.3.4. Isolation of the Maleness factor

Molecular biology could enable GSS to be developed by manipulating the sex determination system. In the medfly, maleness is determined by the presence of particular sequences on the Y chromosome. Through the analysis of Y-chromosomal deletion, we have mapped the Maleness factor to a region on the long arm comprising approximately 15% of the entire chromosome; the distal two thirds of the long arm have no apparent function as corroborated by the existence of the dot-like Y chromosomes mentioned above. Based on this information, the proximal part of the long arm was micro-dissected from mitotic chromosomes. After PCR amplification this material was used for cytological and molecular analyses. Currently, work is in progress to enrich the microdissected material for Y-specific sequences. In collaboration with David Haymer (Hawaii), these will then be used to screen for the Maleness gene(s). The aim is to construct strains where the Maleness factor is controlled by a conditional promoter in such a way that only females are produced if the appropriate induction is applied.

3.2. MEDFLY REARING

3.2.1. Introduction

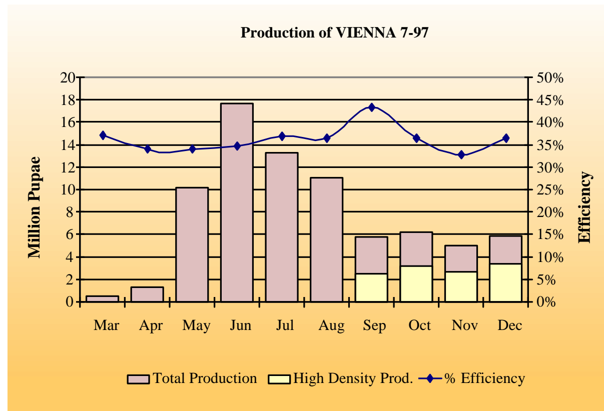
One of the most important tasks that the medfly rearing group performs is the testing, characterisation and definition of the rearing protocols of potential mass rearing strains that come from the genetic sexing group. This important step prior to transferring the technology to operational programmes, allows the different strains to be viewed in terms of the genetic potential for which they were designed. The principles of recombinant filter design, mass rearing protocols and general strain management are essential elements of the transfer of technologies to operational programmes

Research on previous strains including SEIB 6-95, SEIB 6-96, VIENNA 4/Tol-94 and VIENNA 6-94 was concluded and two new strains took their place in mass rearing. One of these strains, VIENNA 7-97 was tested because the translocation used has the potential to remain genetically stable under mass rearing conditions and its investigation was mandatory for future use in international facilities. The strain is based on *wp* and ***tsl*** as selectable markers.

The second strain introduced into mass rearing was AUSTRIA 6-97, a strain based upon the VIENNA 6 translocation but which includes, as well as *wp* and *tsl*, the body colour mutant yellow, *ye*. This third mutation *ye*, enabled the number of triplication males to be assessed during mass rearing (see section 3.1.1.2) and provided an essential tool to study the management of these individuals. The strain will not be used in operational programmes.

In addition to this work, the medfly facilities were upgraded. First, the new extension for larval rearing was inaugurated in mid 1997. The extension is of modern design and has very good environmental controls, well suited to the investigation of mass rearing of genetic sexing strains. The opportunity was also taken to upgrade and modify the older part of the facility.

Figure 3.4: Production of VIENNA 7-97 during 1997. Production characteristics were determined during May-August.



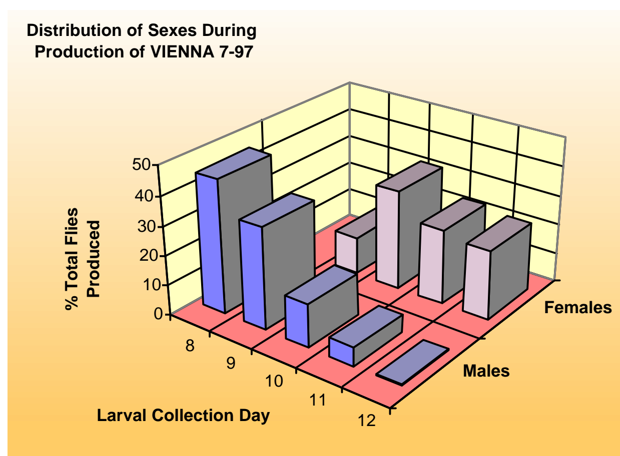
3.2.2. A New Genetic Sexing Strain: VIENNA 7-97

A good genetic sexing strain must have three basic properties, the translocation used must be simple, have low levels of recombination and produce few triplication offspring.

VIENNA 7-97 was first described in 1995 and the breakpoint of the translocation was subsequently mapped and shown to be very close to the *wp* mutation (see Figure 3.2). This strain demonstrates a very low survival of triplication offspring and a low recombination frequency, although recombination is a little higher than for VIENNA 6-94. However, the production of recombinants and their subsequent accumulation during mass rearing is much lower than for VIENNA 6-94. In VIENNA 7-97, triplication females are found in brown pupae. Although the frequency of these females is reportedly low, it can cause an over-estimation of genetic recombination.

In all respects the VIENNA 7-97 has proven to be a very promising temperature sensitive GSS strain for mass rearing. In this report, VIENNA 7-97 is compared with VIENNA 6-94 which was previously the best performing *tsl* GSS in mass production.

Figure 3.5: The production of males and females of VIENNA 7-97, from consecutive daily larval collections. The first larval collection occurs on day 8 of larval development.



3.2.2.1. Production

In order to test and characterise a genetic sexing strain in mass rearing, it is necessary to produce large numbers of flies for at least 3-4 months. This improves the estimate of production parameters for operational mass rearing facilities.

Monthly production of the strain was built up quickly from a small colony of 33,500 pupae produced in the genetic sexing laboratory. Figure 3.4 shows that levels above 10 million pupae per month were produced within two months of receiving the strain and were sustained for four months during which time production performance was assessed. The efficiency of mass rearing (% eggs that become pupae) was maintained relatively constant, which augments the value of the characterisation parameters.

Production was decreased after the characterisation phase, to compare the effects on genetic recombination, of high and low density colonies and high/low female:male ratios in cages. The work will be ongoing for some time, but the production from the two lines is reflected in the figure.

A distinctive attribute of *tsl* based GSS is the bimodal development of sexes during their growth from the moment of egg hatching. This attribute is very marked in VIENNA 7-97, where larvae begin to be harvested after 8

days of development. Males are collected predominantly during days 8 and 9, while females are collected mainly during days 10-12 of larval collection (Figure 3.5), with no females collected in day 8.

3.2.2.2. Genetic stability

VIENNA 7-97 strain has demonstrated good genetic stability; better than VIENNA 6-94. After 11 generations in mass rearing and the production of more than 40 million flies, the VIENNA 7-97 has shown a low and constant level of recombination (Figure 3.6), compared with the previous most stable strain, VIENNA 6-94 (Figure 3.6 insert) over the same number of generations, and only 14 million flies. The production levels are important in this comparison, since it has been observed that higher production rates induce a faster accumulation of recombinants. Clearly, at nearly three times the rate of production, VIENNA 7-97 shows no sign of the so called “genetic breakdown” of previous strains studied. In addition, the estimate of recombination is inflated by the inclusion of triplication individuals.

3.2.2.3. Egg production

Egg production in colonies rearing *tsl* based GSS is a key element to their overall production performance for the following reasons; firstly, the colonies are maintained at slightly lower temperatures (24°C) than for other strains to protect the heat sensitive females against stress and at lower temperatures female medflies oviposit less eggs, secondly, a large number of eggs is required since more than 70% of the eggs are heat treated to kill female embryos, for male-only production and thirdly, the space required for such an adult colony is substantial and this resource is for the most part limited.

VIENNA 7-97 demonstrates a different egg production pattern compared to VIENNA 6-94, although the overall egg production is similar (during a 14 day period: VIENNA 7-97 females produce 12.5 eggs/fem/d, VIENNA 6-94 females produce 12.7 eggs/fem/d). However, VIENNA 7-97 females appear to be more productive over a shorter period. Figure 3.7 shows the different egg production curves for VIENNA 7-97 and VIENNA 6-94 and VIENNA 7-97 tends to produce significantly more eggs during the first week of oviposition compared with VIENNA 6-94, which produces more consistently over 2 weeks.

The VIENNA 7-97 egg laying curve is advantageous in mass rearing, since more eggs are produced early in a ‘cage egg laying cycle’. This means that a cage of VIENNA 7-97 can economically be removed from production earlier than VIENNA 6-94, thereby freeing the critical space resources. This faster turnover of oviposition cages should make the management of VIENNA 7-97 colonies more efficient.

Figure 3.6: Recombination in VIENNA 7-97 during 1997. In continuous mass production generations overlap. Generations are calculated from the majority of production which makes up a generation.

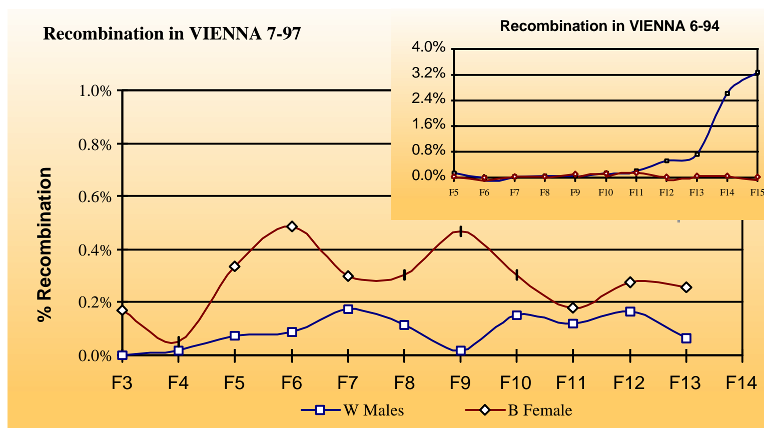


Figure 3.7: Daily egg production from VIENNA 7-97 (egg hatch was 81%) and VIENNA 6-94 (egg hatch was 79%).

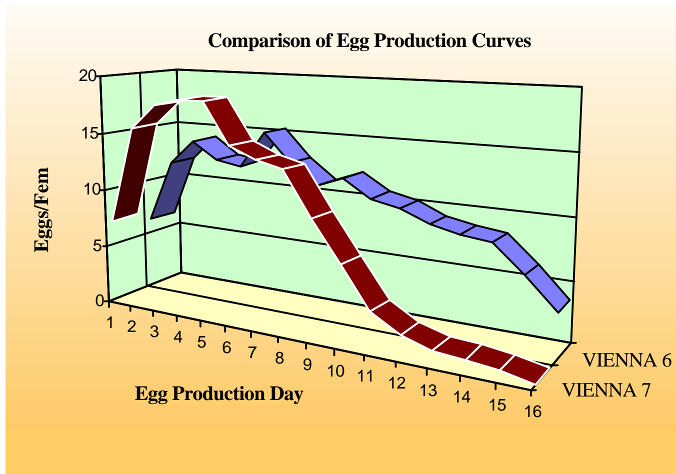


Table 3.7: Adult emergence characteristics in VIENNA 7-97 compared with VIENNA 6-94. Day 1 of consecutive daily pupae collections, correlates to day 8 of larval production.

	VIENNA 7-97		VIENNA 6-94	
	Male	Female	Male	Female
Day 1	91.6	83.9	78.3	68.4
Day 2	86.0	78.2	60.0	71.4
Day 3	66.3	81.1	42.8	72.7
Day 4	35.5	79.4	28.8	69.5
Day 5	15.3	73.3	14.7	60.5

Table 3.8: The flight ability index (a measure of propensity to fly), for VIENNA 7-97 and VIENNA 6-94, for consecutive daily pupal collections.

	VIENNA 7-97		VIENNA 6-94	
	Males	Females	Males	Females
Day 1	94.8		79.6	53.2
Day 2	93.0	70.4	79.9	74.5
Day 3	89.3	91.5	78.4	74.5
Day 4	91.3	89.5	72.9	77.9
Day 5	82.9	88.8	60.8	51.3

3.2.2.4. Emergence

The patterns of emergence of male flies from puparia in *tsl* based GSS are governed by the presence of triplication individuals, the proportion of which increases in consecutive days' larval collections. Figure 3.5 not only shows the bimodal production of males and females from a single egg set, but also that ca 70% of males are produced in the first 2 consecutive larval collections. The emergence of flies from VIENNA 7-97 pupae shows the characteristic decrease in male emergence with consecutive daily collections, similar to all GSS (Table 3.7). However, the % emergence in the two first days of male production are significantly higher than for other *tsl* based GSS. In addition, the emergence quality of females is also much higher, decreasing marginally in later collections (as for VIENNA 6-94). Partial emergence is also reduced in VIENNA 7-97 and an analysis of un-emerged pupae shows a small proportion of identifiable triplicated females (1%) and males (0.2%). These data confirm that the survival of triplication individuals is significantly reduced in VIENNA 7-97.

3.2.2.5. Flight ability

Flight ability, a measure of the proportion of emerged flies that are pre-disposed to dispersal, is one the most important parameters to be maintained in mass reared and released medflies. Characteristically, for strains studied to date, the majority of emerged males are able to fly away and disperse

(Table 3.8) and that the value for this parameter (83.4%) was relatively similar amongst strains. This means that when a male emerges, it has an equal chance of flight, regardless of the strain.

However, the flight ability of VIENNA 7-97 is higher in both sexes compared with VIENNA 6-94 (Table 3.8). In addition, the two primary collections of males (pupae collection days 1 and 2) have flight abilities which tend to be highest. Female flight is greatly increased also, and again in the collections (days 3, 4 and 5).

3.2.2.6. Efficiency of rearing

The efficiency of mass rearing is both economically and qualitatively significant in medfly production. Over the past few years it has been shown that various GSS produce a similar number of pupae from a given number of eggs (i.e. they have similar egg-pupae efficiency of ca 42%) and that the total number of flying males and females are also similar at the end of production (ca 58%).

Table 3.9: Production efficiencies of VIENNA 7-97 compared with VIENNA 6-94.

	VIENNA 7-97		VIENNA 6-94	
	Male	Female	Male	Female
Efficiency 1 (% egg to pupae)	37.1		41.6	
Efficiency 2 (% Pupae to flyers)	68.6	64.3	41.6	59.5
Production Efficiency(Total flyers)	53.3	14.3	36.7	21.3
Flyers, regardless of gender	67.6		58.0	

However, VIENNA 7-97, with its various rearing differences, shows a slightly reduced survival from eggs to pupae (ca 37%; probably because of the loss of triplication individuals early in larval development). This has consequences for seeding rates, because triplication larvae will still consume diet before dying.

VIENNA 7-97 demonstrates much better survival of pupae to fliers (again the lack of triplication individuals) in both sexes (Table 3.9) and it is clear that VIENNA 7-97 produces significantly more males than females (Table 3.9): 3.5 fold more males are produced in total production. This has consequences for the colony management of the strain, but there are benefits in quality of the product for the field.

3.2.2.7. Heat treatments

Temperature sensitive GSS allow female zygotes to be killed following high temperature treatment (34°C) during the egg stage. A high temperature treatment of eggs of these strains should produce the highest potential number of males, with few if any females (ie high accuracy).

The results of studies on *tsl* GSS in 1995/96 showed that the timing of the application of the high temperature treatment during egg (zygote) development has significant effects on the potential number and accuracy of production of males. A treatment known as the “Low-High” treatment was found to be an efficient and generic treatment of eggs from *tsl* GSS.

Table 3.10: The effect on male fly production, when the 34°C heat treatment is applied early in embryogenesis (High-High and High-Low treatments) and late in embryogenesis (Low-High) for VIENNA 7-97 compared with VIENNA 6-94

Treatment	Male Flies VIENNA 7-97†	Male Flies VIENNA 6-94†
High-High (48h 34°C)	3,412.8	3,940.9
High-Low (24h 34°C-24h 23C	6,094.3	4,607.3
Low-High (24h 23°C-24h 34°C	20,223.1	9,580.2
Low-Low (no temp treat)	21,247.5	12,843.7

†: from 82,250 eggs

The application of the high temperature treatment during late embryogenesis (24-48 h after egg collection; the "Low-High" treatment) produces significantly more male flies compared with heat treatments during early embryogenesis (0-24h after egg collection). The Low-High treatment produces a similar number of males as for non-treated eggs, indicating that the Low-High treatment conserved all potential male flies. In addition, the proportion of male flies was nearly 100% from the Low-High treatment. The use of the Low-High treatment in large scale programs will increase the efficiency and the economy of *tsl* GSS production.

This generic treatment has also been confirmed for VIENNA 7-97, however an additional finding is the high recovery (emergence and flight ability) of males in comparison to other *tsl* GSS, such as VIENNA 6-94. Table 3.10 shows that significantly more male flies were produced from VIENNA 7-97, using late-embryogenesis heat treatments or no heat treatment (Low-High and Low-Low treatments). In addition, the number of male flies recovered from the VIENNA 7-97 low-High treatment was nearly equal to the number of males recovered from non treated eggs. The reduced number of triplicated individuals will make the use of VIENNA 7-97 much more acceptable in field programmes, because the efficiency of handling the flies will be greatly enhanced and more cost effective.

In VIENNA 6-94, the proportion of triplication individuals, after the Low-High treatment, increased with consecutive daily larvae/pupae collections, reducing the emergence quality in consecutive collections. In addition to reducing overall quality, it requires a management strategy during rearing to control both the rate of rearing (therefore the inherent quality of consecutive collections) and the auditing or tracking of each collection so that management for releases could be enhanced.

VIENNA 7-97 does not show this characteristic (Table 3.11). Emergence quality is not a problem, with emergence in consecutive daily collections being nearly equal. This consistency in product and high quality (equal to normal strains) is desirable in pupae management strategies.

The efficiency of rearing only males in VIENNA 7-97 is markedly higher than for VIENNA 6-94 for the Low-High treatment (Table 3.12). In broad terms, the key element here is the proportion of eggs that become males. First, nearly 25% of VIENNA 7-97 eggs become males and second, these males are all balanced (normal) males. Theoretically, 25% of all eggs represent balanced males in GSS and VIENNA 7-97 is the first GSS to demonstrate this level of recovery, un-contaminated by triplication individuals (unbalanced males).

Table 3.11: Emergence of flies from puparia after the Low-High treatment for VIENNA 7-97 compared with VIENNA 6-94, for 3 consecutive daily collections of pupae.

Strain	VIENNA 7-97			VIENNA 6-94		
Larval Collection	1	2	3	1	2	3
Emergence from the 'Low-High' Treatment	95.8	96.3	89.4	86.9	77.5	58.4

Table 3.12. Production of pupae and male flies from eggs, resulting from the different heat treatments of eggs of VIENNA 7-97 compared with Vienna 6-94.

Treatment	VIENNA 7-97 % Egg to brown pupae	VIENNA 7-97 % Egg to male flies	VIENNA 6-94 % Egg to brown pupae	VIENNA 6-94 % Egg to male flies
High-High	5.2	4.1	6.7	4.8
High-Low	9.4	7.4	8.5	5.6
Low-High	25.6	24.6	18.2	11.6
Low-Low	29.9	25.8	26.9	15.6

Pupae from each treatment were produced from 82,250 eggs.

3.2.3. The Yellow Body Strain and the Management of Triplication Individuals

The strain used in these studies is based on VIENNA 6-94, but with an additional body colour mutation (*ye*). As explained in section 3.1.1.2 the inclusion of this mutation enables the fate of triplication individuals to be followed. In mass rearing, the strain is called AUSTRIA 6-97 and females have a yellow body as well as being *wp* and *tsl*. Male triplication individuals will also be yellow body whereas normal males will have the wild-type body colour. In this way the appearance of triplication individuals can be audited in consecutive collections of pupae.

It is clear from the data that there is a large proportion of triplication individuals in the pupal production of GSS when the VIENNA-6 translocation is used. Table 3.13 shows that nearly 24% of pupae are triplication individuals and only 1.9% of these emerge as adults. The remaining 76% are balanced (normal) males, from which 93.6% emerge as adults. The impact of triplication individuals on VIENNA 6 type GSS is to 'virtually' reduce the quality of production, while the balanced product (that which is effective in the field) is actually of high quality but masked by the low emergence of the triplication individuals.

Table 3.13: The proportion of balanced and triplicated individuals produced from a single seeding and the respective emergence of males from brown puparia of AUSTRIA 6-97.

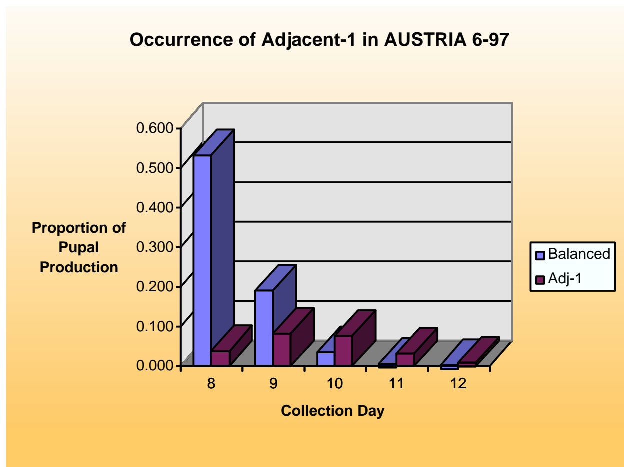
% Balanced Males	% Triplication Males	% Emergence from Balanced Males	% Emergence from Triplication Males
76.3	23.7	93.6	1.9

The management of triplication individuals is therefore going to play a significant role in the interpretation of quality of pupal production for VIENNA 6 type strains. Triplicated individuals have always been assumed to be the cause of decreasing emergence from consecutive pupal collections. Table 3.14 shows that this is true: if emergence was calculated regardless of body colour, then it should resemble the VIENNA 6-94 data (shown). Indeed there is little difference between the two data sets. However, when we look at the division of emergence qualities between balanced and triplication individuals, it can clearly be seen that balanced

Table 3.14: Emergence rates from puparia containing balanced individuals and triplicated individuals in AUSTRIA 6-97 compared to that of VIENNA 6-94.

Collection	Comparison from V6-94 Males	Emergence regardless of body colour	Emergence of balanced individuals	Emergence of triplicated individuals
Day 1	78.3	77.7%	93.4%	0.8%
Day 2	60.0	61.7%	95.8%	2.2%
Day 3	42.8	29.4%	85.0%	2.7%
Day 4	28.8	12.2%	89.1%	1.5%
Day 5	14.7	6.2%	80.3%	0.3%

Figure 3.8: The occurrence of triplicated and balanced pupae during consecutive daily pupae collections of AUSTRIA 6-97



individuals emerge at much higher rates than triplication individuals, and that the emergence rate of balanced individuals is consistently high amongst all collections.

The occurrence of triplication individuals in pupal collections must therefore explain the decreasing emergence rate observed in the VIENNA 6-94 strain. Figure 3.8 shows that the majority of triplication individuals are produced in latter collections.

This work is ongoing and will explore the effect on survival of triplication individuals from male-only production (after eggs are heat treated) and in other quality parameters such as flight ability and longevity. The management of triplication individuals appears to be related to temperature control during larval rearing, another aspect to be explored.

3.2.4. Genetic Sexing Strains and the International Scene

Over the past several years, GSS have been placed into 5 out of 8 international medfly facilities with operational programmes (Table 3.15). The recombinant filter rearing system, designed in Seibersdorf to control genetic and production qualities, is now in four of those facilities. Argentina has continued to demonstrate significant suppression of wild medflies using its GSS. Guatemala also continues to demonstrate a high efficacy of releasing genetic sexing males in field comparisons with normal strains. Chile and Madeira are only now beginning to release genetic sexing males into the field.

The medfly rearing group provides much technical support for all the programmes indicated which use a GSS. This unique support is essential to the programmes and places a high level of responsiblity on the group.

Table 3.15: Major medfly facilities in the world and the strains of medfly reared in them.

Principle World Facilities	Strain	Filter	*Millions Produced*
Argentina (Mendoza)	SEIB 6-96	Yes	250-300
Chile (Arica)	SEIB 6-96	Yes	2-3
Greece (Crete)	SEIB 6-96	No	3-5
Guatemala (El Pino)	VIENNA 4/Tol-94	Yes	120-150
Guatemala (El Pino)	Antigua (Bi Sexual)	No	200-300
Mexico (Metapa)	Tapachula (Bi Sexual)	No	500-600
Portugal (Madeira)	VIENNA 6-96	Yes	10-15
USA (Hawaii CDFA)	Hawaii (Bi Sexual)	No	100-120
USA (Hawaii USDA)	Maui (Bi Sexual)	No	250-300

3.3. MEDFLY SEXUAL BEHAVIOUR

As a supportive technology to the use of SIT against medfly, mating behaviour studies were initiated in 1995. One of the main goals was to design a reliable quality control test to assess the field mating competitiveness of GSS for use in field releases. Another goal was to assess the mating compatibility of wild medfly populations from many parts of the world. The absence of any incompatibility would enable a particular GSS to be used in many facilities and remove the need for repeated strain construction

Like most of the tephritid species, the sexual behaviour of medfly is complex; the acceptance by a female of a male partner is based on chemical, visual and physical cues. For SIT programmes, it is of major importance that the mating behaviour of mass reared insects remains similar to that of the wild flies. Mass reared medflies must exhibit the same behavioural pattern as the wild individuals so that, once released, they will compete successfully for wild insect mates.

3.3.1. Assessment on Field Cage Host Trees of Sexual Compatibility and Mating Competitiveness

3.3.1.1. Cage set-up

Field cages have been already used with success to assess the mating competitiveness of various tephritid species. However, different methods were applied in the past and comparative analysis was difficult. In 1997, an international group standardised the sexual compatibility and mating competitiveness assessments of medfly mass reared strains. Cylindrical field cages of Saran® screen (20x20 mesh), with flat floor and ceiling, 2.9 m in

diameter and 2.0 m in height are used. Each cage is supported by a PVC-frame. As the objective is to create conditions similar to those of an orchard, each field cage is set up over a medfly host tree which fills as much as possible of the inner volume of the cage. When necessary, a black or green shading material can be added on the top of the cage to avoid the “green-house” effect. Temperature, relative humidity and light intensity are recorded every 30 min. in order to provide additional information on the correlation between environmental conditions and mating performance. When the equipment is available, the air pressure can also be recorded.

3.3.1.2. Biological material

Wild pupae are obtained from infested fruits collected in the location where field releases are planned. For the assessment of mating performance, mass reared pupae must be irradiated one to two days before emergence, according to the standard protocol of the rearing facility. Male and female flies are kept in separate cages (and preferably rooms) from emergence and provided with water and protein-based food. No later than two days before testing, flies are marked on the notum with water-based paint to be able to distinguish the type of flies during the test. Flies must be tested at the age of sexual maturity (9-14 day old for wild, 4-7 for mass reared flies).The testing period must cover the peak of maximum daily sexual activity. Consequently, when no preliminary information is available, the test is started at dawn and covers most of the day (seven consecutive hours is a minimum requirement). Depending on the type of test, flies are released as described in Table 3.16 In both types of test, males are given about 30 min. to establish their territory before the females are released.

Table 3.16: Description of the tests

		Number of Flies Released per Cage			
		Wild		GSS	
		9 to 14d. old		*4 to 7d. old*	
Type of Test	To Measure				
Unisexual	Mating competitiveness	30	30	30	0
Bisexual	Sexual compatibility	30	30	30	30

The number and type of calling males are censused every 30 min., starting from female release, and the type and location of mating pairs are checked on a continuous census. Following mating, the pairs are removed and kept in a glass (or plastic) vial, and duration of mating is recorded. The success of the mating can be confirmed by dissecting the female spermathecae to detect the presence of sperm.

3.3.2. Indices Assessed

The *Proportion of Mating* (PM), measures the participation of the flies in mating during the tests and reflects the suitability of the test conditions (see Table 3.17). It represents the overall mating activity of both wild and mass-reared flies. If the PM<0.2 then the data should not be further analysed as the conditions of the test were unsuitable.

Mating competitiveness is measured using the *Relative Sterility Index* (RSI) (see Table 3.17). The RSI is the proportion of wild females mated by mass reared males. The index ranges from 0 (no matings between wild females and mass reared males) to +1 (all matings of wild females carried out by mass reared males) through an equilibrium at +0.5 (wild females are mated in equal proportion by mass reared males and wild males).In the case of GSS (where only males will be released in the field), it is believed that mating competitiveness measured in a unisexual test (see Table 3.16) is more representative of the field situation.

Table 3.17: Indices (W for wild, L for mass reared fly; first letter, second letter)

To Measure	Index	Range of Values min	equilibrium	max
Proportion of Mating	$PM = \frac{\text{No of pair collected}}{\text{No of female released}}$	0	+0.5	+1
Sexual compatibility	$ISI = \frac{(WW + LL) - (WL + LW)}{(LL + WW + LW + WL)}$	-1	0	+1
Relative Performance	$MRPI = \frac{(LW + LL) - (WL + WW)}{(LL + WW + LW + WL)}$	-1	0	+1
Relative Performance	$FRPI = \frac{(WL + LL) - (LW + WW)}{(LL + WW + LW + WL)}$	-1	0	+1
Mating competitiveness	$RSI = \frac{LW}{(LW + WW)}$	0	+0.5	+1

Sexual compatibility between a wild population and a mass reared strain is measured by the *Isolation Index* (ISI) (see Table 3.17). It ranges from -1, negative assortative mating, where all matings occur between partners of opposite strains (as found in some *Drosophila* species) to +1, positive assortative mating, where all matings occur within the strains. A value of 0 represents random mating where an equal proportion of the four possibile mating types occurs.

The *male and female relative performance indices* (MRPI and FRPI respectively) (see Table 3.17) enable the ISI, to be better analysed in terms of male and female contribution. These two indices range between -1, where all matings are carried out by mass reared males (or females) to +1, where all matings are carried out by wild males (or females), through an equilibrium at 0, where equal numbers of mating are carried out by wild and mass reared males (or females).

3.3.3. Field Cage Tests Carried Out in Support of Action Programmes

Within the framework of SIT action programmes, the standard field cage assessment can be used as a decision tool in the following ways:

i. to decide which strain is the most suitable candidate for release, prior to initiation of the programme,
ii. to monitor the mating competitiveness of the released flies during the implementation of the programme,
iii. to decide when a strain should be replaced

In 1997, technical support was provided for 3 countries participating in SIT programmes: Argentina, Crete (Greece) and Israel. During these field trips, the methodology was fine-tuned and transferred to the local teams. It has now been adopted as a routine quality control test in these countries.

3.3.3.1. Argentina

For several years, predominantly male releases from the SEIB 6-96 GSS, mass reared in KM8 rearing facility in Mendoza, Argentina, have proved to be very efficient in controlling medfly in that Province. In 1997, the authorities of the Patagonia Region, decided to initiate an SIT programme to eradicate medfly from their area using flies provided by Mendoza. It was therefore necessary to assess the compatibility of males of the SEIB 6-96 strain with flies from Patagonia and a series of tests were carried out in San Miguel de Tucumán (Tucumán Province) from 24 March till 11 April 1997 with the aim to assess:

i. the sexual compatibility of SEIB 6-96 sterile flies with the wild Patagonian population:
ii. the mating performance of SEIB 6-96 sterile males when competing with wild Patagonian males for wild female mates

iii. the effect of an increased sterile to wild males ratio (5:1) on the wild population.

The results are summarised in Table 3.18 and they show that the participation of the flies in the mating process was satisfactory (PM=±0.50) indicating that the test conditions were suitable. The SEIB 6-96 strain and wild Patagonian population are sexually compatible (ISI=0.309) and SEIB 6-96 and wild Patagonian males were equivalent in mating performance (RPI=0.089), however SEIB 6-96 females were more willing to mate (less choosy) than wild females (RPI=0.368) when competing with wild males. SEIB 6-96 sterile males achieved one quarter of wild female mates (RSI=0.264) and in a 5:1 sterile to fertile male ratio, SEIB 6-96 males largely overcome the increased mating performance of wild males (RSI=0.636).

Since it was shown that SEIB 6-96 was suitable for SIT programme in the Patagonia Province, sterile males from this strain have been released weekly since September 1997.

3.3.3.2. Crete

Sterile males of SEIB 6-96 reared in the facility of the University of Crete (Voutes, Heraklion) were used successfully in an SIT pilot test to control medfly in the Valley of Fodele (Crete). Despite these good results in the field, field cage tests done locally in 1996 indicated a poor performance of the strain. A series of field cage tests was conducted from 21 till 31 July 1997 at Forteza (Crete) to re-assess the quality of SEIB 6-96.

Table 3.19: Field cage tests in Forteza, Crete Island (variance in *italics*)

Type of Test	PM	RSI	ISI	RPI	RPI
	Mean Values of the Indices				
Bisexual Control Wild	0.667	0.375	0.071	-0.214	-0.143
1 replicate 28 pairs	*n.a.*	*n.a.*	*n.a.*	*n.a.*	*n.a.*
Bisexual	0.471	0.121	0.242	-0.536	-0.001
3 replicates 71 pairs	*0.009*	*0.044*	*0.005*	*0.181*	*0.026*
Unisexual	0.810	0.176			
1 replicate 17 pairs	*n.a.*	*n.a.*			

Only a few replicates were found suitable for analysis and the results in Table 3.19 show that despite a good overall participation of the flies in mating (see PM), most of the matings were achieved by wild flies.

SEIB 6-96 and wild population from Crete are sexually compatible (ISI=0.242) but the male relative performance was in favour of wild males (RPI=-0.536), when the relative performance of wild and SEIB 6-96 females was equivalent (RPI=-0.001).

When competing with wild males, SEIB 6-96 sterile males achieved only 17% of wild female mates (RSI=0.176). In addition, on two consecutive cloudy days, SEIB 6-96 males did not mate with wild females (data not shown).

Based on these data and on the quality of the flies seen in the laboratory (poor emergence, very low flight ability), it was

Table 3.18: Field cage tests in San Miguel de Tucumán, Argentina (variance in *italics*)

Type of Test	PM	RSI	ISI	RPI	RPI
	Mean Values of the Indices				
BISEXUAL	0.488	0.332	0.309	0.089	0.368
18 replicates 520 pairs	*0.019*	*0.047*	*0.034*	*0.052*	*0.025*
UNISEXUAL 1:1	0.492	0.264			
21 replicates 304 pairs	*0.037*	*0.027*			
UNISEXUAL 5:1	0.509	0.636			
6 replicates 81 pairs	*0.025*	*0.064*			

concluded that the minimum quality requirements for testing flies in field cages were not reached. Considering that the flies used in the field cages were treated differently from the flies released in the field, it was concluded that the results were due to the bad quality of the specific batch of individuals tested and no conclusion was drawn on the quality of the SEIB 6-96 strain.

3.3.3.3. Israel

The Israeli authorities intend to start a pilot eradication programme in the Arava Valley as a prelude to a much larger regional medfly SIT programme. The Israeli authorities have stipulated that only males can be released and since no medfly rearing facility will be built in Israel, two suitable sources of sterile males were identified; the Camacha rearing facility (Madeira Island) which produces VIENNA 6-96, and the El Pino facility (Guatemala) which produces VIENNA 4/Tol-94. Shipments of sterile male pupae were sent from these two locations to Israel. Because no wild flies were available at the time of testing, flies from a laboratory-adapted strain colonised from wild pupae one year before the tests (CMBLab) were used in the tests. The field cage tests were run from 1 till 11 September 1997 in Bet Dagan (Israel) to assess the relative mating competitiveness of VIENNA 6-96 and VIENNA 4/Tol-94 males and the effect of shipment on the behavioural quality of the flies.

Results presented in Table 3.20 show that there was excellent participation of the flies in mating (PM>0.5) for both GSS, (not significantly different according to Tukey's LSD test, P=0.05). However there was a significant difference between the mating competitiveness of males from the two strains. VIENNA 6-96 males achieved about 40% of matings of females (RSI=0.390) compared to 24% for VIENNA 4/Tol-94 males (RSI=0.236).

On the basis of the relative performance of males from these two strains, and taking into account the laboratory QC data, it was concluded that VIENNA 6-96 GSS was the most suitable candidate for SIT programme against medfly in Israel. The programme will start in 1998 with sterile VIENNA 6-96 male pupae being shipped from Madeira. It is likely that a transportation problem resulted in the poor performance of the flies from Guatemala.

3.3.3.4. Seibersdorf

As no female medflies, even if sterilized, could be used in Israel for field cage tests due to quarantine reasons, the assessment of sexual compatibility between GSS and wild flies from Israel was carried out in green house in Seibersdorf. The tests were run in June 1997 with two GSS strains available in the Entomology Unit: VIENNA 4/Tol-94 and VIENNA 7-97, and Israeli flies that emerged from wild pupae shipped to Austria.

Data presented in Table 3.21 show that the participation of the flies in mating (PM) was satisfactory, despite a slightly lower participation when VIENNA 7-97 flies were tested. There was no evidence of any sexual incompatibility between either of the GSS and flies from Israel (ISI values of 0.230 and -0.062). The male relative performance differed for the two strains with males from VIENNA 4/Tol-94 performing better (RPI=0.227) than males from VIENNA 7-97 (RPI=-0.492) but in both cases GSS females were more

Table 3.21: Green house tests in Seibersdorf, Austria
(variance in *italics*)

Type of Test		Mean Values of the Indices				
		PM	RSI	ISI	RPI	RPI
Bisexual VIENNA 4/Tol-94						
	3 replicates	0.532	0.510	0.230	0.227	0.436
	78 pairs	*0.003*	*0.014*	*0.001*	*0.026*	*0.024*
Bisexual VIENNA 7-97						
	3 replicates	0.391	0.246	-0.062	-0.492	0.208
	56 pairs	*0.006*	*0.008*	*0.095*	*0.010*	*0.137*

Table 3.20: Field cage tests in Bet Dagan, Israel
(variance in *italics*)

Type of Test	Mean Values of the Indices PM	RSI
Unisexual Control CMBLab		
5 replicates	0.473 b	0.347ab
54 pairs	*0.025*	*0.036*
Unisexual VIENNA 6-96		
16 replicates	0.634 a	0.390a
287 pairs	*0.011*	*0.020*
Unisexual VIENNA 4/Tol-94		
8 replicates	0.649a	0.236 b
121 pairs	*0.004*	*0.013*

willing to mate (less choosy) than wild females (RPI=0.436 and 0.208 for VIENNA 4/Tol-94 and VIENNA 7-97 respectively).

The mating competitiveness values which are also given for information (since it would be more relevant in unisexual test) showed a two fold difference between males of the two GSS, with a clear advantage for VIENNA 4/Tol-94 males (see RSI).

These results were not surprising since the VIENNA 7-97 strain was not specifically constructed for field use whereas VIENNA 4/Tol-94 had been specifically backcrossed to field flies. It was concluded that of these two strains, VIENNA 4/Tol-94 represented the most suitable candidate for SIT programme against medfly in Israel. This result is somewhat at variance with the tests carried out in Israel.

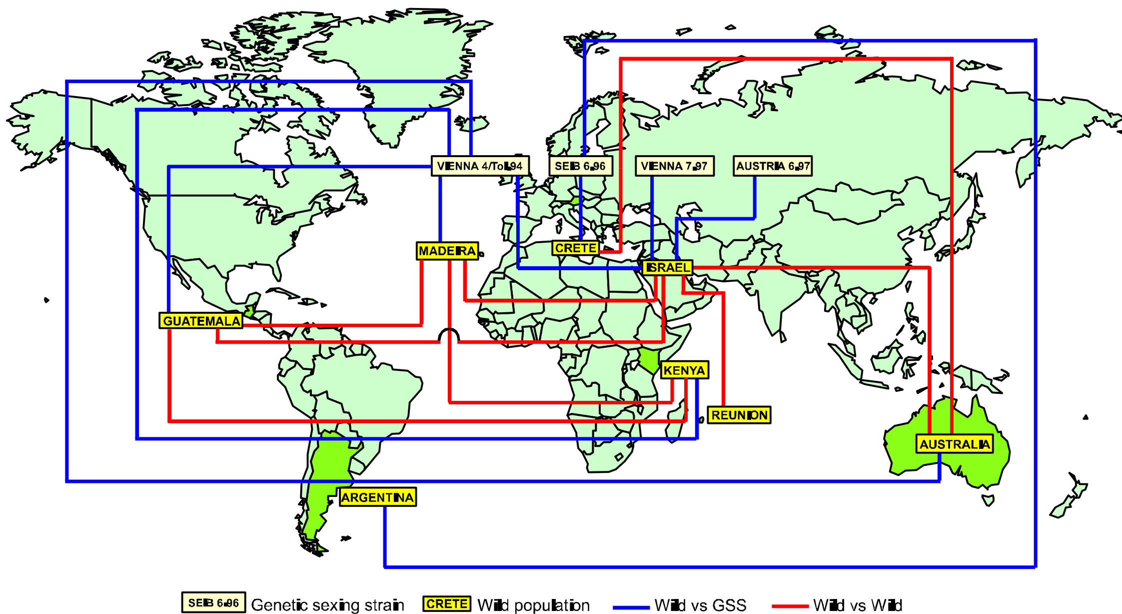
3.3.4. Assessment of Sexual Compatibility Among Medfly Populations from Different Origins

In SIT programmes using standard bisexual strains, colonies for mass rearing are established by collecting pupae from the field. As GSS are constructed in the laboratory, it is much more difficult to establish new strains and the GSS used in SIT programmes are generally backcrossed with a wild population to increase the genetic variability before they are transferred to a facility. In some cases it has proved impossible to perform this type of backcross. The wild population used for backcrossing is in some cases different in geographic origin from the target wild population. This situation has raised concerns about the sexual compatibility of these strains with wild medfly populations in different countries. In order to promote the use of GSS in medfly SIT programmes with the aim to provide a strain which can be used in many parts of the world, it became important to know if there are mating incompatibilities among different medfly populations.

Most countries involved in SIT programmes have strong quarantine restrictions which avoid the importation of wild flies and the laboratories at Seibersdorf represented the most suitable location for these types of experiment. Medfly is already present in Europe and its importation to Austria is not restricted by quarantine regulations.

The standard field cage test described above was carried out in a temperature controlled green house (3x5x2 m, containing 6 citrus trees) as in Seibersdorf local environmental conditions were for most of the time, not suitable for field experiments with medfly. The tests, started in April 1997, assessed the sexual compatibility between different wild medfly populations and the sexual compatibility between wild populations and GSS strains. The wild flies used were shipped as pupae to Seibersdorf and the emerging adults were used in the test.

Figure 3.9: Cross mating tests between wild populations, and wild populations and GSS



The following question was posed at the beginning of the tests. Have medfly populations worldwide evolved pre-mating isolation barriers? If the answer is no then a competitive GSS should be effective world-wide and any reduction in competitiveness would be a reflection on the quality of the strain.

3.3.4.1. Sexual compatibility among wild populations

Wild insects were received as pupae from 7 locations representing 5 continents: Guatemala, Madeira (Portugal), Crete (Greece), Israel, Kenya, Reunion (France) and Australia and the flies were tested at the age of sexual maturity following the protocol described for bisexual type experiments (see above). The tests lasted for 7 hours, starting at dawn, 8 combinations were tested (see Figure 3.9), representing a total of 27 replicates and 669 mating pairs and the overall results are presented in Table 3.22. The overall results showed that about 40% of the possible matings occurred (PM=0.403), confirming that the environmental conditions in the green house were suitable for the tests.No sexual isolation was found among the different wild populations (ISI=0.234) and no qualitative differences were found among the populations tested. However, some mating "preferences" were found among the strains as shown by RPI and RPI values of 0.377 and 0.293, respectively. This means that the four types of mating did occur but with different proportions (quantitative differences).

3.3.4.2. Sexual Compatibility Between Wild Populations and Genetic Sexing Strain

Wild medfly from 7 countries (Guatemala, Argentina, Madeira Island (Portugal), Crete Island (Greece), Israel, Kenya and Australia) were tested with four GSS available from the Entomology Unit (VIENNA 4/Tol-94, SEIB 6-96, VIENNA 7-97 and AUSTRIA 6-97). 8 types of comparisons were tested (see Figure 3.9) as described above, representing a total of 37 replicates and 1,095 mating pairs.

The overall results presented in Table 3.22 show that, regardless of the type of combination tested, about 50% of all possible matings occurred (PM=0.488) and no sexual isolation was found between the different wild populations and the GSS (ISI=0.225). The mating performance of wild and GSS males, regardless of the type of females is equivalent (RPI=-0.003) and the GSS females are less

Table 3.22: Cross mating tests (variance in *italics*, * based on absolute values)

Type of Comparison	PM	RSI	ISI	RPI	RPI
	Mean Values of the Indices				
WILD vs WILD					
8 comparisons	0.403	/	0.234	0.377 *	0.293 *
27 replicates	*0.010*	/	*0.088*	*0.050*	*0.031*
669 pairs					
WILD vs GSS					
8 comparisons	0.488	0.349	0.225	-0.003	0.285
37 replicates	*0.017*	*0.051*	*0.040*	*0.099*	*0.063*
1095 pairs					

choosy than their wild counterparts in selecting a mate partner (RPI=0.285).

The overall mating competitiveness of GSS males compared with various wild populations is highly satisfactory (RSI=0.349).

3.3.4.3. Preliminary conclusions and future experiments

It is obvious that all possible combinations of wild medfly populations from the countries where medfly is present cannot be reasonably tested. However, the locations chosen and tested to date are representative of the distribution of medfly in the world, with the exception of Hawaii.

As no case of sexual isolation was found during these experiments among wild populations, it can be concluded that wild flies from different origins exhibit similar sexual behaviour and are compatible. Consequently, backcrossing of GSS can be based on any type of wild material from any geographic origin.

This comparative assessment of wild and GSS insects confirms the previous conclusions. The sexual compatibility value (ISI) is similar to that obtained between wild populations and confirmed that GSS flies have not evolved specific mating behaviour patterns. GSS males showed high mating competitiveness with all types of wild insects tested and any lack of competitiveness of GSS flies can only reflect a decrease of the quality of the flies produced.

To complete the analysis, it is intended to add to the comparative assessment of wild populations flies from South Africa and Hawaii in 1998.

3.3.5. Characterisation of GSS sexual behaviour

Because GSS are constructed following a specific crossing scheme, questions were raised about the possibility of the development of specific sexual behaviour. The concerns were somewhat laid to rest by the results presented above. Nevertheless, various tests were run in 1997 after a literature review on the data available for normal bisexual strains.

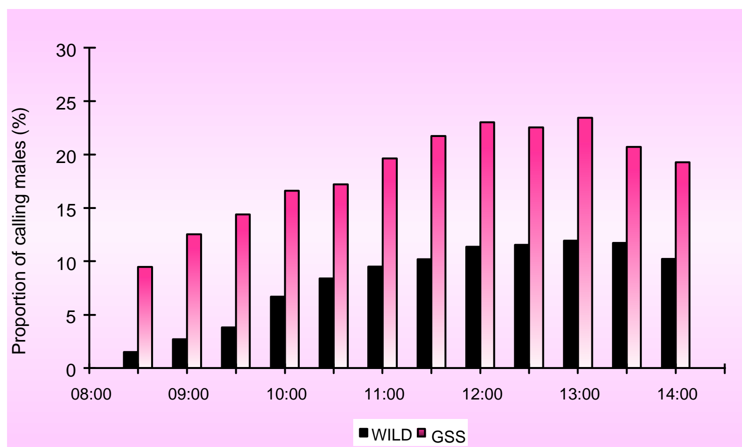
3.3.5.1. Mating competitiveness

It was shown in the previous section that, when a one GSS to one wild fly ratio is tested, GSS males obtain about one third of the wild female mates (see Table 3.22). In SIT programmes, this slightly reduced competitiveness (when compared to one half if wild and GSS males would show the same competitiveness) of GSS males is largely overcome by increasing the sterile to fertile ratio.

3.3.5.2. Emission of pheromone

The emission of pheromone by the males, or “male calling behaviour”, plays an important role in the attraction of females to mate. In field cage experiments, it was found that a higher proportion of GSS males than wild males were calling throughout the day. Figure 3.10 presents data obtained in Argentina with SEIB 6-96 males in unisexual type test. Since calling activity represents a high investment of energy, GSS males waste much of their resources.

Figure 3.10: Proportion of GSS and wild calling males in field cages during the day



3.3.5.3. Spatial distribution of matings in the host tree

In nature, wild females visit leks (aggregation of calling males) and select within the most suitable lek, the most suitable partner for mating. Mating takes place at the original location where the male was calling. Being in the best located lek for a male increases his chances to be selected by a wild female. In field cage tests, it appears that most of the WW matings occurred in the top of the tree, whereas most of the GSS male/wild female (LW) matings were distributed over the different parts of the host plant. Figure 3.11 presents data obtained in unisexual type field cage tests in Argentina. It can be concluded than GSS males are not as selective as wild males regarding the location of the lek they join.

3.3.5.4. Duration of mating

It is believed that the duration of mating reflects the amount of sperm and accessory fluid transferred by the male to the female. Data obtained in a bisexual type field cage test are presented in Figure 3.12 The duration of mating of GSS males is significantly shorter (Tukey's HSD test, $P<0.05$) than those of their wild counterparts. This could suggest that GSS males do not elicit the correct post-mating response in wild females.

3.3.6. Comparative Sound Analysis of the Love Songs of Sterile and Fertile GSS Males

It was shown that the characteristics of male love songs during the medfly courtship play a major role in female acceptance of a male to mate. In order to mate successfully with wild females, sterile GSS males must exhibit the same pattern of love songs.

A small project was initiated with a cost-free intern, Michaela Gruber, in October 1997 to assess the effect of sterilisation on the characteristics of the male medfly love songs. The objective is to compare, through video and sound recording, sterile and fertile VIENNA 7-97 male songs when courting fertile VIENNA 7-97 females, together with their relative mating success. The complete analysis will be available in 1998.

Figure 3.11: Original location of mating pairs within a field-caged host tree

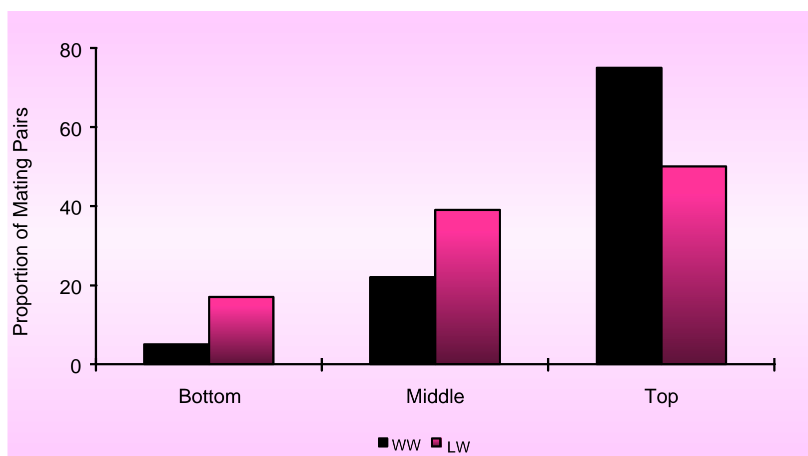
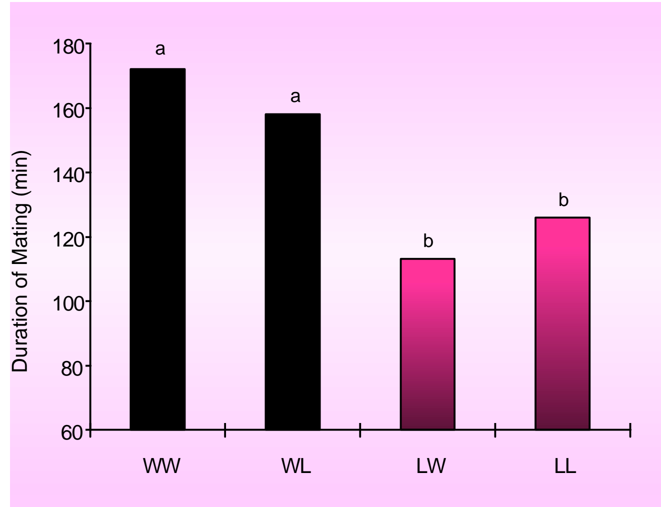


Figure 3.12: Duration of mating (W for wild, L for mass reared fly)



4. TRAVEL

Staff member	Destination	Period of absence	Purpose of travel
Cayol, J.-P.	Tucuman, ARG	97-03-22 - 97-04-14	Implementation of medfly field cage tests
	Crete, GRE	97-07-20 - 97-08-01	Implementation of medfly field cage tests
	Israel, ISR	97-08-31 - 97-09-19	1) Implementation of medfly field cage tests 2) Acting Scientific Secretary for RCM on Medfly Mating Behaviour
Fisher, K.	Queensland, AUL	97-01-14 - 97-01-21	Invited expert to Science Advisory Panel on eradication of *B. papayae*
	Mendoza, ARG	97-03-15 - 97-03-30	Installation of recombinant filter system
	Crete, GRE	97-07-02 - 97-07-09	Provide advice on the rearing of GSS SEIB 6 96
	Madeira, POR	97-07-10 - 97-07-18	Technical support for rearing of GSS VIENNA 6-96
	Queensland, AUL, Perth, AUL	97-07-28 - 97-07-30	1) Consultant on SIT facility design for *B. papayae* 2) Advise on the use of GSS in Pilot Project in Australia
	Mendoza (ARG), Arica (CHI), Guatemala City (GUA), Tapachula (MEX)	97-10-04 - 97-10-26	1) Technical support for the implementation of GSS 2) Present lectures at an Agency Medfly Training Course
	Madeira, POR	97-11-16 - 97-11-22	1) Installation of the recombinant filter system 2) Present lectures at an Agency Medfly Training Course
Franz, G.	Guatemala City, GUA	97-07-05 - 97-07-13	1) Participate in the RCM on Medfly Genetic Sexing 2) Advise on the relevant genetics of GSS in Guatemala
	Varna, BUL	97-09-20 - 97-09-27	Attend the European Drosophila Research Conference
Opiyo, E.	Tanga, URT	97-02-24 - 97-02-28	Scientific Secretary for Tsetse Automation RCM
	Maputo, MOZ	97-09-27 - 97-10-05	Presentation at the 24th meeting of the ISCTRC OAU/IBAR
	Tanga, URT	97-11-15 - 97-11-19	Present lectures at an Agency Tsetse Regional Training Course
	Nashville, TN, USA	97-12-12 - 97-12-20	Presentation at the Annual Conference of Entomological Soceity of America
Robinson, A.S.	Addis Ababa Axum, ETH	97-02-01 - 97-02-16	1) Scientific Secretary for Tsetse Genetics RCM and 2) Presentation at Int. Study Workshop "Integrated Rural Development through Sustainable Management of Tsetse and Trypanosomosis: The Way Forward"
	Asilomar, CA	97-05-08 - 97-05-13	Presentation at "Second International Workshop on Transgenesis of Invertebrate Organisms"
	Guatemala City, GUA	97-07-05 - 97-07-12	Scientific Secretary for Medfly Genetic Sexing RCM
	Hyderabad and New Delhi, IND	97-08-16 - 97-08-27	1) Presentation at the "Second Global Meet on Parasitic Diseases, Hyderabad. 2) Organize a workshop on mosquito SIT. 3) Visit the Malaria Research Center in New Delhi
	Tanga, URT	97-12-06 - 97-12-14	Present a lectures at an Agency Tsetse Regional Training Course.

5. PUBLICATIONS AND PRESENTATIONS AT INTERNATIONAL MEETINGS

CAYOL, J.P., BUYCKX, E.J., LOUSSAIEF, F., ZARAI, M., BOUKHARI, M., ARFAOUI, T., Control of Mediterranean fruit fly with Vienna-43/44 *tsl* sterile males in Tunisian oases, Proc. of IOBC/WPRS Int. Open Mtg - Working Group “Fruit Flies of Economic Importance”, Lisbon, Sept. 1997.

FISHER, K., Irradiation effects in air and in nitrogen on Mediterranean fruit fly (Diptera:Tephritidae) pupae in Western Australia. J. Econ. Entmol. 90: 1609-1614, (1997)

FRANZ, G., WILLHOEFT, U., KERREMANS, Ph., HENDRICHS, J. RENDON, P., Development and application of genetic sexing systems for the Mediterranean fruit fly based on a temperature sensitive lethal mutation. In “Evaluation of Genetically Altered Medflies for Use in Sterile Insect Technique Programmes” IAEA Proceedings of the final Research Co-ordination Meeting, Clearwater, Florida, 11-13 June, 1994, IAEA, 85-95

GOODING, R.H., FELDMANN, U., ROBINSON, A.S., “Care and maintenance of tsetse colonies”, The Molecular Biology of Insect Disease Vectors (CRAMPTON, J., BEARD, C.B., LOUIS, C., Eds), Chapman Hall (1997) 41-56.

OPIYO, E., LUGER, D., FELDMANN, U., HENDRICHS, J., PAAREL, A., Development in tsetse fly mass rearing at Seibersdorf Laboratories, ISCTRC, Moputo, Mozambique (1997).

OPIYO, E., LUGER, D., FELDMANN, U., HENDRICHS, J., PAAREL, A., Status of tsetse fly mass rearing at Seibersdorf Laboratories, Entomological Society of America, Nashville (1997).

ROBINSON, A.S., “The potential role of the International Atomic Energy Agency in the development of a sterile insect programme for mosquitoes”, 2nd Global Meet on Parasitic Diseases, Hyderabad 1997 (abstract).

ROBINSON, A.S., “Improvement in the automation of tsetse fly mass rearing”, Integrated Rural Development Through Sustainable Management of Tsetse and Trypanosomosis/Mosquito and Malaria,. Addis Ababa, Ethiopia (1997).

ROBINSON, A.S., “Genetic sexing strains of the medfly, *Ceratitis capitata*: Lessons for mosquitoes”, 2nd Global Meet on Parasitic Diseases, Hyderabad, India (1997).

ROBINSON, A.S., FRANZ, G., “Biological constraints to the use of transgenic insects for pest control”, 2nd International Workshop on Transgenesis of Invertebrate Organisms, Asilomar, California, USA (1997).

WILLHOEFT, U., Fluorescence *in situ* hybridization of ribosomal DNA to mitotic chromosomes of tsetse flies (Diptera: Glossinidae: Glossina), Chromosome Research 5:262-267 (1997)

WILLHOEFT, U., MUELLER-NAVIA, J., FRANZ, G., Analysis of the medfly sex chromosomes by microdissected DNA probes, Genome (in press).