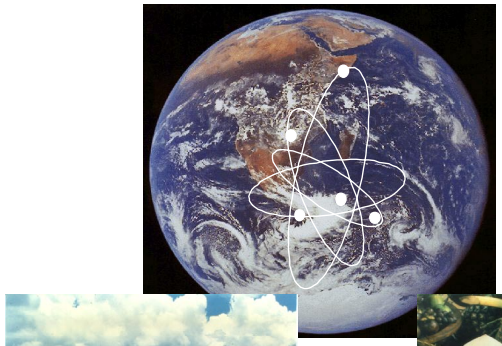
ANNUAL REPORT 2001

ENTOMOLOGY UNIT
FAO/IAEA Agriculture and Biotechnology Laboratory
Seibersdorf

Table of Contents

1. Introduction



The use of genetic sexing strains (GSS) for operational medfly SIT programmes is now firmly established with all new facilities being designed to rear these strains. Despite the widespread uptake of this technology, significant improvements in terms of stability and productivity are still possible. In 2000 an inversion was introduced into a particular GSS both to improve stability and to take advantage of the particular production profile of a strain. Initial results in 2001 demonstrated the improvement of stability and higher production and it is planned to transfer this strain to operational facilities midway through 2002.

Genetic markers for released sterile flies can be very important in solving problems related to unexpected outbreaks in release areas. They can answer the question as to whether an outbreak originated from a release of non-sterilized flies or a new wild population has become established. In 2001, mtDNA markers were used to answer this question crucial question for the medfly SIT programme in South Africa.

In tsetse, use of the TPU 3 rearing system was expanded and is now in routine use for the maintenance of the G. pallidipes colony and the system will be used in new rearing facilities in Africa. Several important tsetse species are distributed over many countries in Africa, sometimes with discontinuous distributions. For areawide SIT it will be most efficient if one colony of a particular species can be used over the whole distribution of the species. This would require that there are no barriers to mating between the sub-populations and preliminary field cage evaluations have been carried out. The initial results indicate that there are no pre-mating isolation barriers between geographically distinct populations of the same species.

There were no major changes in staffing or the infrastructure of the Unit during the year. In order to maintain activities at the level of previous years it has been necessary to increase attempts to obtain extrabudgetary funds. This has been successful with funds being provided by the University of California, Riverside and the Moscamed programme in Guatemala.

1.1 The olive fly, *Bactrocera oleae*

In 2001 a start was made on establishing a colony of the olive fly, *Bactrocera oleae*. The insect is the key pest of olives and was identified during the sub-programme review as a good candidate for the development of SIT. Studies on rearing in the past indicated that certain constraints would need to be overcome before an efficient mass rearing system could be developed. A first attempt to rear a colony was successful for two generations only, providing an appropriate larval diet will be the main target for activities in 2002.

1.2 Medfly transformation

Using funds from a successful competitive grant application a consultant has been recruited to expand activities related to transgenic medfly. These activities were carried out in close collaboration with Dr. A. M. Handler from USDA/ARS, Florida. Many new transgenic lines marked with a green fluorescent protein have been induced and are undergoing evaluation for stability and productivity. In addition, several lines expressing a red fluorescent protein have been isolated. The goal of this work is to provide medfly strains to SIT programmes that have a visible genetic marker. Some initial studies on monitoring the fitness of these lines have shown that a transgene can have a negative effect on some life table parameters.

1.3. Field cage developments

The use of the field cages for the evaluation of sterile fly quality continues to grow for both tsetse and medfly. In tsetse, they have been used to demonstrate compatibility between different populations of the same species, to test the effects of radiation in air and nitrogen and the effects of chilling the males. In medfly they have been used to assess the competitiveness of GSS strain carrying a phenotypic marker and to monitor the effects of kairomones on male attractiveness to females. The use of these compounds to improve the competitiveness of sterile males could have a major impact on the effectiveness of fruit fly SIT programmes. The work was carried out by Dr. T. Shelly, USDA/ARS, Hawaii, during a consultancy.

1.4 Sperm use and its identification

In tsetse, work reported last year showed that the hybrid sterility between different species may be a possible option for field intervention. DNA analysis of the progeny of females mated with both males of their own species and males of a second species have shown that in some cases the female is able to make a choice as to which type of sperm has been used. Rather surprisingly, this depends on the sequence of with which the matings were made. Sperm use will be very important if this concept is to have any application in the field.

During a consultancy of Dr. T. Chapman, Univ. London, initial experiments were started to develop DNA based methods to identify the origin of sperm in mated females. This would enable the mating status of trapped wild females to be determined in terms of whether they mated with a sterile male or a wild male. Further work needs to be done to refine this approach and make it suitable for application in field programmes.

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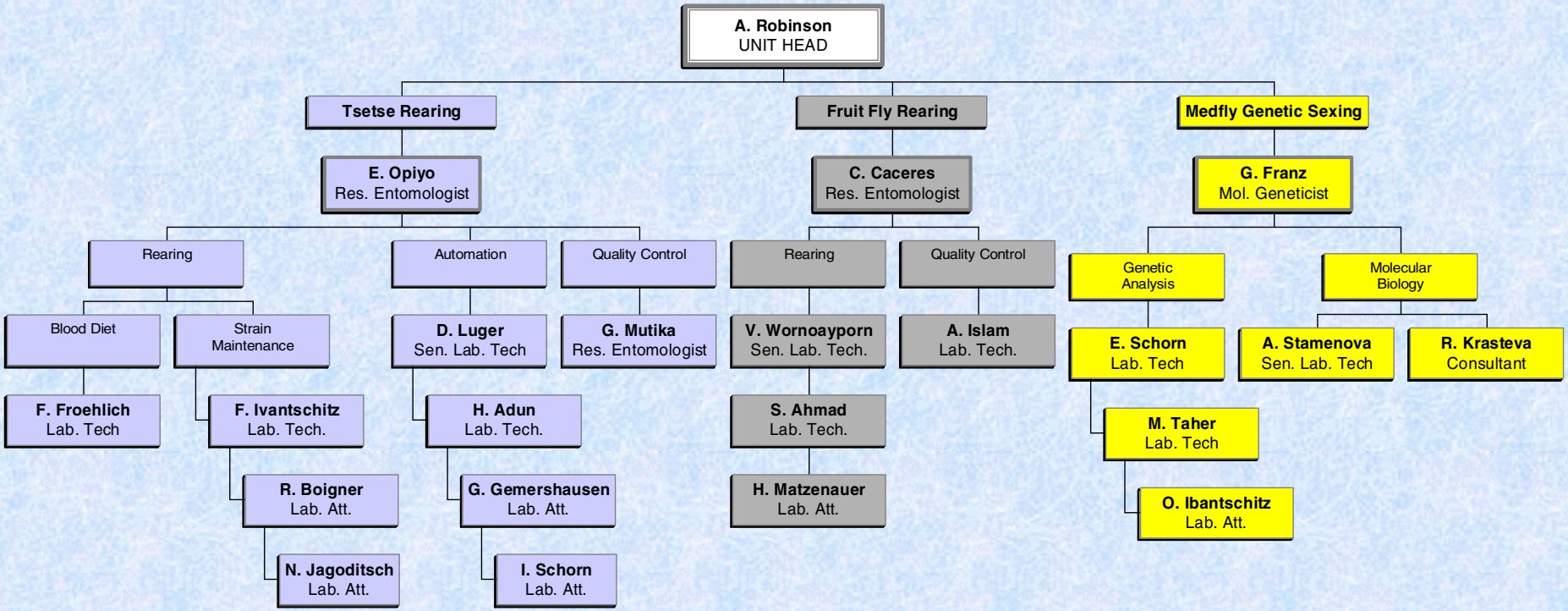


2. Quality Control and Hybrid Studies in Tsetse

Quality control procedures for tsetse are being standardised to ensure that mass production of tsetse flies in Africa can be developed in a coordinated fashion. The field cage studies reported here are an integral part of this and this technology will be transferred to several field projects during 2002. The field cage can also be used to assess the level of mating compatibility between different populations, or even species, of tsetse. Building on the experience in medfly experiments have been carried out with different geographic populations of the same species to see if it will be necessary to develop separate colonies from each area for a single species. This would impose considerable constraints on the operation of SIT programmes. The limited tests so far carried out show that there is no pre-mating isolation barrier between different populations of two species.

Large-scale aerial release of sterile tsetse flies will probably require the use of a chilled release system and initial experiments have been carried out to assess the effect of different chilling procedures on the fitness of adult male tsetse. Long periods of cold have a detrimental effect on fly survival and the time that the flies spend at low temperatures will have to be minimized. The concept of containerized rearing systems for tsetse has now been developed and a prototype will be constructed and delivered in 2002. A containerized system can only make sense when combined with the TPU3 mass rearing system.

In the Annual Report for 2000 the possibility of using hybrid sterility for tsetse control was re-evaluated. These studies were completed in 2001 by examining the use of sperm in mutiple mated hybridized females. It was demonstrated that female tsetse appear to be able to choose the type of sperm for fertilization when they have been mated with two different males.

The provision of a suitable blood diet to tsetse for large colonies remains a concern. Currently a large radiation source is required to reduce the bacterial load in the blood. This type of facility cannot be found everywhere, especially in some areas in Africa where tsetse rearing facilities will be established. In 2001 some work was initiated with non-radiation based decontamination i.e. pasteurization and this will be continued in 2002.

2.1 Mating Compatibility of Tsetse Strains

The sterile insect technique (SIT) is being integrated into several tsetse fly control programmes in regions where rearing facilities are not yet established and flies from other regions may have to be used were colonies have already been established. In addition the establishment of regional mass-rearing facilities is being proposed. It is thus important to investigate biological implications of these developments on the mating compatibility to reassure stakeholders that it is not necessary to colonize strains of the same species from different geographical locations. The goal is to be able to use a single strain of one species over the whole range of that species. This philosophy has been very successfully applied to the medfly. If a strain can be used effectively in different geographical regions this will do away with the need for a rearing facility in each area or the maintenance of different strains in one facility for use in different areas. To assess the possibility of using this strategy, investigations of the mating compatibility of two *G. pallidipes* strains from Ethiopia and Uganda and two *G. m. centralis* strains from Botswana (still in the process of laboratory adaptation) and Tanzania (colonized for several generations in Kenya) were carried out. The mating tests were conducted under natural light in a walk-in field cage erected inside a greenhouse. Temperature and humidity were monitored using an OPUS® electronic thermo-hygrograph and light intensity at three different levels inside the cage was measured by a TES-1334 Lightmeter®.

2.1.1 G. pallidipes

The *G. pallidipes* strain from Uganda was originally colonized in 1975 while colonization of the Ethiopian strain started in 1996. Adult *G. pallidipes* of the Uganda strain were obtained from the SSPC procedure. The virgin flies from the two strains were collected and kept in normal colony conditions until the day of the test. Males from one strain were marked with a dot of polymer paint on the notum. Males were thirteen days old and females eight days old on the day of the test. Twenty males from each of the two strains were released into the field cage immediately followed by twenty virgin females starting at 10:00h local time. Any fly that dropped to the ground at the time of release was considered a non-flyer and replaced. Mating pairs were immediately collected into single tubes and the time mating started was recorded in minutes from initial release. The duration of mating was recorded in minutes. The pairs that were removed were not replaced. The flies that mated were kept overnight and females dissected to determine insemination rate as well as spermathecal fill. Both males and females were also checked for salivary gland hypertrophy regardless of mating status. The mean environmental conditions in the cage were 23.7°C to 27.4°C, 30.3% to 35.1% RH and 993.7hPa to 994hPa. Pressure remained constant through the two hours of the test but varied negligibly with day, humidity dropped from start to end of each test and temperature rose from start to end.

Table 1: Pre-mating period (min.), mating duration (min.) and mean spermathecal values of *G. pallidipes* females from Ethiopia mated with males from Ethiopa (E) or Uganda (U).

	Pre-mating period		Mating Duration		Spermathecal Value	
Male	**E**	**U**	**E**	**U**	**E**	**U**
No.	13	45	13	45	13	43
Mean	42.2	58.1	33.8	23.9	1.5	1.4
s.e.	8.4	4.9	2.1	0.6	0.1	0.1
Min.	11	5	25	17	0.5	0
Max.	115	120	49	32	2	2

The data is presented in **Table 1**. Mating propensity ranged from 0.2 to 0.4 on each day the test was run. Several unsuccessful mating attempts during any one test were recorded without any attempt having been made

to differentiate between strains. In these cases, the female flexed her abdomen, closed the wings or flew away to avoid mating. Spermathecal values for females mated with either strain were homogeneous (P=0.27; df=1,54; F=1.23) but duration of mating was significantly longer for males from the Ethiopia strain (P=0.00; df=1,56; F=35.43). In one out of 10 replicates all matings were exclusively between individuals of the Ugandan strain. There was a high incidence of salivary gland hypertrophy among males and females from the Ethiopian strain. All males of both species that engaged in mating had normal salivary glands whereas 52.8% and 8.5% of the Ethiopia and Uganda strains respectively, that did not mate had salivary gland hypertrophy. Of the females that mated 43% had salivary gland hypertrophy and a similarly high proportion (59.5%) of those that did not mate were infected. It can be concluded that there is no absolute barrier to mating between males of the Uganda strain and females of the Ethiopia strain. However the problem associated with salivary gland hyperplasia will need to be addressed.

2.1.2 G. m. centralis

The *G. m. centralis* strain from Tanzania has been reared in the laboratory for several generations while laboratory adaptation of the Botswana strain collected in the field started in 1999. In the first test, males from each of the Tanzania and Botswana strains were used in mating tests with females from the Botswana strain. In the second test, males from each of the Tanzania and Botswana strains were used in mating tests with females from the Tanzanian strain. The flies were kept in normal colony conditions until the day of the test. Males from one strain were marked with a dot of polymer paint on the notum. Males were ten days old and females three days old on the day of the test. Ten males from each of the two strains were released into the field cage immediately followed by ten virgin females starting at 10:00h local time. The release process lasted about five minutes. Any fly that dropped to the ground at the time of release was considered as a non-flyer and immediately replaced. Mating pairs were collected into single tubes and a record made of the time in minutes (pre-mating period) from initial release for each case. The flies were kept overnight and the females dissected to determine insemination rate as well as spermathecal fill. The mean environmental conditions were similar to those reported above.

Table 2: Pre-mating period (min.), and spermathecal values of Tanzania *G. m. centralis* females mated with Botswana (B) or Tanzania (T) males.

	Pre-mating period		Spermathecal Value	
Male	**B**	**T**	**B**	**T**
No.	6	24	6	24
Mean	32.7	40.5	0.4	0.8
s.e.	8.0	7.1	0.2	0.1
Min.	3	7	0	0.25
Max.	55	120	0.5	1.5

The results are shown in **Tables 2 and 3**. Spermathecal values for females mated with either strain were significantly different (P=0.0067; df=1,29; F=8.54). Duration of mating was in excess of two hours for both strains. In the first test, mating propensity ranged from 0.2 to 0.7. Several unsuccessful mating attempts during any one test were recorded without any attempt having been made to differentiate between strains. Males from

Table 3: Pre-mating period (min.) and spermathecal values of Botswana *G. m. centralis* females mated with Botswana (B) or Tanzania (T) males.

	Pre-mating period		Spermathecal Value	
Male	**B**	**T**	**B**	**T**
No.	4	11	4	11
Mean	56	63.18	1.87	1.27
s.e.	22.6	12.42	0.12	0.14
Min.	19	10	1.5	0.5
Max.	113	120	2	2

the Botswana strain were generally weak. In the second test, the pre-mating period was not significantly different for the two strains (z-test: P(Z<=z)=0.4). Spermathecal values for females mated with the males from Botswana strain were significantly higher than for females mated with males from the Tanzania strain (z-test: P(Z<=z)=0.0007). Duration of mating was in excess of two hours for both strains, exact observations for duration were not done. Mating propensity ranged from 0.11 to 0.62 on each day the test was run.

Mating and flying activity occurred in the cage and feeding behaviour was exhibited by infrequent probing of the investigator. Males flew about in the cage while the females generally remained in one position. Mating occurred on the roof, walls and zip of the cage, stem and leaves of the plant. Four pairs successfully engaged genitalia while flying and then landed on the plant or cage. Males from the Botswana strain were generally weak but still managed to fly and participate in mating activity. The general weakness is inferred from the fact that out of the fifteen males collected at emergence up to five were dead on the day of the test or unable to fly on release. Insemination rate was 100% although spermathecae were not completely filled with the sperm and seminal fluid transferred. In all the four replicates it was evident that there is no barrier to mating between the two strains. Males from the Tanzania strain successfully mated with females from the Botswana strain and out-competed the Botswana males. However, mating was certainly not random (**Table 4**). In the tests with the *G. pallidipes* females from Ethiopia, significantly more of the Ugandan males were successful (calculated z=10.77, critical one tailed value 1.64). For both tests with *G. m. centralis* females from Botswana and Tanzania, the *G. m. centralis* Tanzanian males were more successful, (calculated z=5.19, critical one tailed value 1.64 and calculated z=4.74, critical one tailed value 1.64, respectively). These observations need to be repeated, as the overall quality of the flies from the different populations was not standardised. The use of one mass reared strain for many different target populations will be very advantageous for tsetse SIT.

Table 4: Mating success of males from different *G. pallidipes* and *G. m. centralis* populations

	Males							
	Ethiopia (*Gp*)		**Uganda (*Gp*)**		**Tanzania (*Gmc*)**		**Botswana (*Gmc*)**	
Females	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Ethiopia (*Gp*)	45	80	13	20				
Tanzania (*Gmc*)					24	80	6	20
Botswana (*Gmc*)					11	70	4	30

2.2 Effect of Chilling on Performance of Adult *Glossina pallidipes*

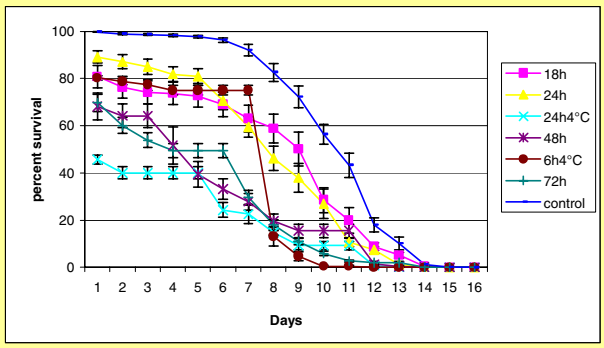
It is envisaged that a chilled adult release system will be implemented in future tsetse SIT programmes on mainland Africa, thus it is necessary to accumulate relevant data on handling procedures for pupae and adults. The effect of low temperature on puparia was the subject of an earlier investigation (Ann. Rep. 2000) and this has now been extended to adults. Male *G. pallidipes* puparia from the SSPC (Ann. Rep. 1999) were used. The flies were kept in standard colony conditions until transfer to 7°C for 18, 24, 48 or 72h. They were then placed at 4°C for 6h and then returned to 23-24°C. The transfer to low temperature was done when flies were seven days old, thus the flies would have been fed at least twice depending on the day that they emerged. Control flies were not chilled.

2.2.1 Stress test

On removal from 4°C the flies were not fed again and they were placed in standard colony conditions without being offered a blood meal. A total of 12-15 cages with 82±12 flies per cage were used for the chilled flies. Six cages with 83±8 flies per cage were used as a control for each treatment. The number of flies surviving was recorded daily and the average survival based on the number of dead flies calculated. The observation continued until all the flies died. This test gave an indication of fat reserves in flies and would reflect on survival of flies when food sources are scarce. It is also important to release flies that will survive for a few days in the wild in search of mates.

First day mortality among flies exposed to low temperature was significantly higher (F=18.94; df=4,80; p=0) than the control group depending on the length of time the adult flies were exposed to 7°C (**Figure 1**). Mortality was 20% for 18h storage, 12% for 24h storage, 32% for 48h storage and 30% for 72h storage compared to less than 1% for flies that were not exposed to low temperature. Separation of means partitioned first day mortality into three homogenous groups, (1) storage at 7°C for 18h or 24h, (2) storage at 7°C for 48h or 72h and (3) control flies. However 50% mortality was recorded in four days for flies exposed for 48h and 72h whereas for exposure for 24h it was eight days, for 18h it was nine days and for control it was ten days.

Figure 1. Survival of adult *G. pallidipes*, without a blood meal, after treatment at different temperatures.



2.2.2 Mating test

Male tsetse from treatments 2 (7°C for 24h) and 3 (7°C for 48h) were mated a day after return from 4°C to 7 day old females. On the day of the experiment the flies were not fed. 10-15 colony females were placed in 10cm diameter round cages and the same number of males from treatments 2, 3 and control flies were introduced and left in the cages overnight. The males were later discarded and all females were dissected and examined 24 hours after mating to determine if insemination had taken place and estimate the amount of sperm in the spermathecae. Insemination rate was over 86% for control males whereas females mated with males exposed for 24h and 48h to 7°C had much lower insemination rates (62 and 37% respectively). Spermathecal fill was correspondingly lower: control; 1.1, 24h; 0.89 and 48h; 0.49. Spermathecal fill was significantly lower for females mated with males stored at 7°C for 24h or 48h (F=10.6; df=3,328; P=0).

In addition to the previous observations on pupal storage at low temperature this information provides the framework within which a chilled adult handling protocol can be developed to improve tsetse SIT. Storage of adults at low temperature beyond 24h may be undesirable because of the higher first day mortality figures and low insemination rate. Although it may not be a routine procedure to stockpile adult flies in a chilled state, the data presented shows that short-term storage of adults may be used, if required. The exact protocol in terms of temperature and time will depend on the production and release logistics developed during the field programmes. For the chilled adult system for aerial release, the sterile males will need to be stored at

about 4°C for the duration of the release period. Storage of adults at low temperature invariably reduced survival and insemination potential of the males. It is thus recommended to expose adult males to cold for the minimum possible time so as not to impair insemination ability and/or mating activity.

2.3 Irradiation of *G. pallidipes* in Nitrogen

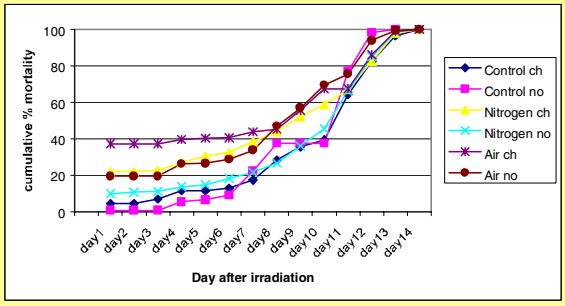
Irradiation in the absence of oxygen reduces the amount of induced biological damage and in some insects a net advantage has been demonstrated when using a nitrogen atmosphere during irradiation. Six days after emergence males that had been fed twice were irradiated in a Co^{60} gamma source. Flies were irradiated with 120Gy in air after chilling at 4°C. For irradiation in nitrogen, flies were chilled and placed in a cylindrical 130cc polycarbonate jar and a steady flow of nitrogen was passed into the jar for five minutes. The jar was then sealed using adhesive tape and the flies irradiated with 180Gy. Dose rate varied from 25-26Gy/minute.

In the first test mature male puparia were incubated for 24h or 72h at 15°C and then transferred to 26.5°C for eclosion. Six days following eclosion, males in each treatment and control flies were split into three groups. One group was irradiated in air with 120Gy, the second group was irradiated in nitrogen with 180Gy and the third group was not irradiated. The groups were further split into two subgroups. In one sub-group the irradiated adults were exposed to 4°C for six hours and returned to the holding room overnight. In the other sub-group the adults were not chilled. This was to simulate a chilled aerial release. The flies were not fed again. Mortality was checked every morning until all flies died. In the second test, puparia were not chilled and six days after eclosion one group of males was irradiated in air with 120Gy, the second group was irradiated in nitrogen with 180Gy and the third group was not irradiated. One subgroup from each treatment was exposed to 4°C for six hours and returned to the holding room overnight. The flies were not fed again. Mortality was checked every morning until all flies died.

2.3.1 Survival

Some of the data is shown in **Figure 2** and it is clear that chilling of the adults has a detrimental effect on their survival and this effect was much greater than the effects of the radiation per se. There was no apparent protection afforded when the irradiation was carried out in nitrogen. The mean overnight mortality was 0.2±0.2 % for control flies, 3.2±1.6% for males irradiated in air and 14.6±4.3% irradiated in nitrogen, the % of non-flyers was 2.4±0.6%, 3.2±1.1% and 7.1±4.6% respectively.

Figure 2. Mortality of adult *G. pallidipes* after irradiation in air and nitrogen



2.3.2 Mating activity

To assess the effect of radiation and chilling on mating competitiveness thirty females were released into the field cage, 5 minutes later thirty males irradiated in air and thirty control males that were not irradiated were released in one cage. In a second cage, thirty males irradiated in nitrogen and thirty control males that were not irradiated were released at the same time. The irradiated males had been chilled at 4°C for 6h. The pre-mating time was taken as the period from the end of release of

males to the time when a successful engagement of genitalia occurred. The time when each successful mating pair was seen was recorded and the pair immediately collected into a tube. The tubes were individually labeled and observed to record time when the mating pairs separated. Duration of mating was then calculated as the difference between pre-mating and end time in minutes. The total number of pairs collected each day was expressed as a proportion of the total possible pairs to give the mating propensity.

The pre-mating period was not significantly affected by the treatment given to the males and it averaged 66.1±3.2min for the control, 68.0±3.8min for males irradiated in air and 60.5±7.7min for males irradiated in nitrogen. Mean spermathecal values were 1.1±0.1 for control, 0.94±0.06 for females mated with males irradiated in air and 0.90±0.08. Mean spermathecal values for females, pre-mating time and duration of mating were not significantly affected by treatment of males ($F=1.68$; $df=2,202$; $P=0.189$) and the environment ($F=1.95$; $df=1,202$; $P=0.164$) but varied significantly on a day to day basis ($F=6.09$; $df=10,202$; $P=0$). It must however be noted that spermathecal fill was lower than expected for the control flies probably due to the age of the flies used. Even though a few spermathecae were full, many flies failed to transfer sperm that completely filled the spermathecae.

The combination of irradiation and chilling induced levels of first day mortality that are probably unacceptable, there was however significant variation among samples. There may be other factors in the handling procedures that require further refinement before large scale adoption of chilled adult release for tsetse flies. Even though published work supports the view that irradiation in a nitrogen atmosphere is beneficial in terms of quality of flies the procedure still remains to be adopted in a practical field programme. The incomplete filling of spermathecae by males that are irradiated and chilled requires further investigation.

2.4 Hybrid sterility: Analysis of Offspring of Females Mated with Two Different Males

Studies reported in the Ann. Rep. 2000 described experiments on hybrid sterility in tsetse and showed that in certain crosses a high degree of sterility could be generated and that females could not always discriminate in mating between males from their own species and non-specific males. These studies have been followed up others assessing whether females that are fertilised by males from two different species show any preference in sperm utilization. In order to do this it was first necessary to identify species specific molecular markers that could be used to ascertain paternity in progeny from multiply mated females.

2.4.1 Species specific markers

Oligonucleotide 10-mer primer set #100/1 from University of British Columbia (UBC) were used in a RAPD based PCR assay to identify molecular markers specific for *G. m. morsitans, G. m. centralis* and *G. swynnertoni.* Eighty primers were tested on DNA from male

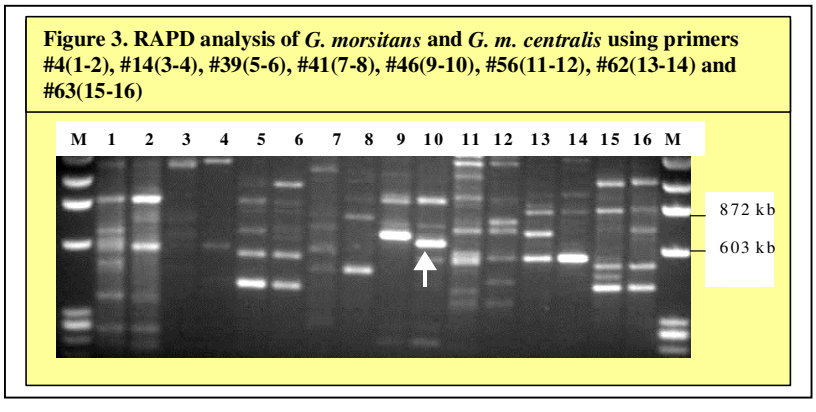
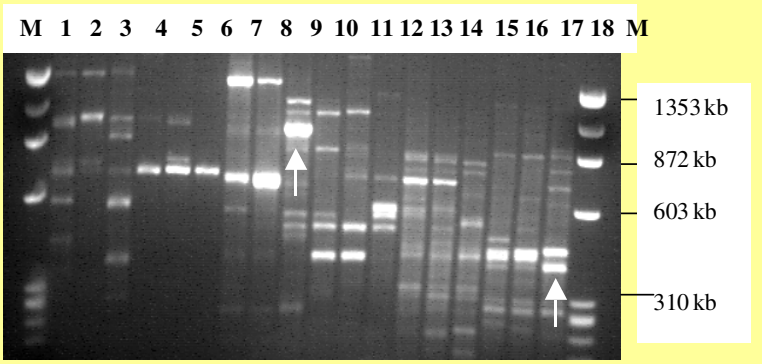


Figure 3. RAPD analysis of *G. morsitans* and *G. m. centralis* using primers #4(1-2), #14(3-4), #39(5-6), #41(7-8), #46(9-10), #56(11-12), #62(13-14) and #63(15-16)

G. m. morsitans, G. m. centralis and *G. swynnertoni* but only 28 were selected for further analysis. It was not easy to identify species specific bands and only diagnostic bands that were intense and highly consistent were used for the paternity analysis in the offspring of multiple mated females. **Figure 4** shows PCR amplification products of *G. m. morsitans, G. m. centralis* and *G. swynnertoni* genotypes with some selected primers. Primer #53 produced intense monomorphic 1353bp and 657bp bands (lanes 7 and 8) for *G. m. morsitans* and *G. m. centralis* that differentiated them from the intense 996bp band produced by *G. swynnertoni* (lane 9). The same primer also produced a less intense but consistent 603bp band in *G. m. morsitans* that could be used to differentiate it from *G. m. centralis*. Primer #66 differentiated between the species with a 427bp (lane 16 and 17) and a 369bp band (lane 18) but was also used to confirm the differences between *G. m. morsitans* and *G. m. centralis* at a 471bp band (lane 16) which was only present in *G. m. morsitans.* Primer #46 (**Figure 3**) also produced intense profiles that could be used to differentiate *G. m. morsitans* from *G. m. centralis*. *G. m. morsitans* produced an intense 711bp band (lane 9) different from the 657bp band (lane 10) produced by *G. m. centralis.*

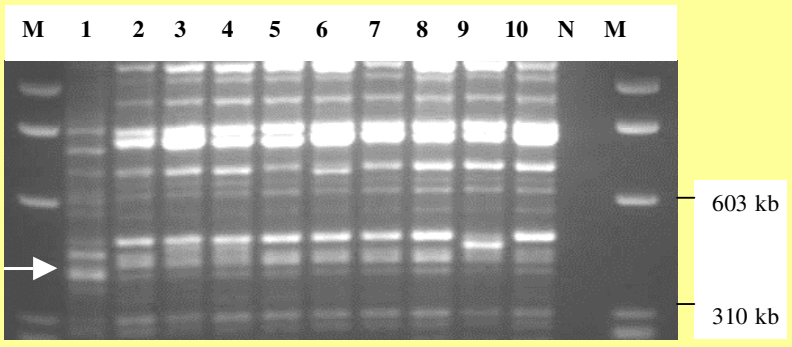
Figure 4 RAPD analysis of *G. m. morsitans, G. m. centralis* and *G. m. swynnertoni* using primer #7(1-30, #27(4-6), #53(7-9), #63(10-12), #65(13-15) and #66(16-18).



2.4.2 Characterisation of offspring from multiple mating

In the RAPD PCR analysis of paternity, the PCR products from the amplification of DNA of both parents and all the offspring were ran on gels and the presence or absence species specific bands were identified in the progeny. It was usually necessary to use data from several different primers to ascertain with certain the paternity of a group of offspring. An example of this is shown in **Figures 5** and **6**.

Figure 5. RAPD analysis using primer #66 from female *G. m. morsitans* (10) mated with *G. swynnertoni* (1) and then with *G. morsitans* (2). The F1 offspring are from 3-9.



Here the genotype of the offspring obtained using primer 66 is shown when females of G. *m. morsitans* were first mated with *G. swynnertoni* and then *G. m. morsitans* males. All the 8 F1's appear to have been fathered by

the second mating, *G. m. morsitans,* and not from *G. swynnertoni.* A similar conclusion can be drawn when primer #53 was used.

Using a combination of markers the paternity of the mutilply mated females as shown in **Table 5** was assessed. This table summarises the data of the complete paternity analysis. *G. m. morsitans* females use sperm from their own males following multiple mating with *G. swynnertoni* and *G. m. morsitans* males, independent of the order of mating. However, when *G. m. morsitans* females are multiply mated with *G. m. morsitans* males and *G. m. centralis(T)* males, sperm use depends on the order of mating. If the first male is *G. m. morsitans* then all progeny are sired by this male and there is no sperm displacement but if the first male is *G. m. centralis(T)* then mixed progeny are produced following some sperm displacement. This latter pattern is also found with *G. m. centralis(T)* females multiply mated with *G. m. morsitans* and *G. m. centralis(T)* males.

Figure 6. RAPD analysis using primer 53 of offspring from G. m. morsitans (11) first mated with G. swynnertoni (1) and then with G. m. morsitans (2) males. The F1 offspring are in 3-10. N=negative control.

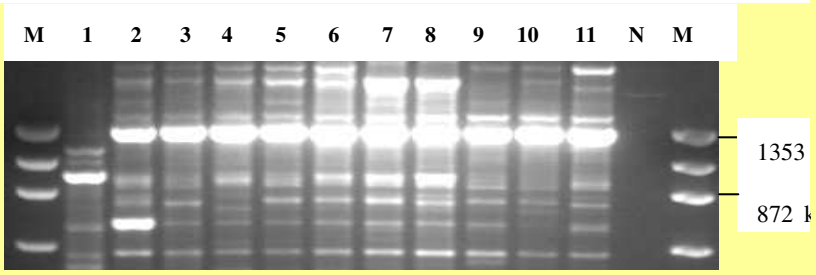


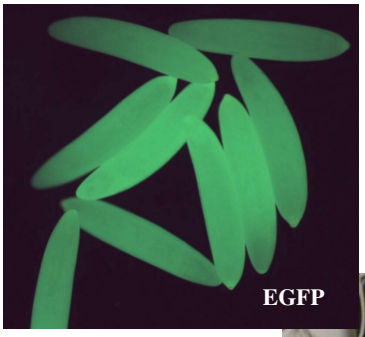
Table 5: Sperm use in tsetse by females mated with males from two different species

Mating sequence[1]	Sperms from 1st male	Sperms from 2nd male	Total
Gmm* x *Gmm/Gs	9	0	9
Gmm* x *Gs/Gmm	0	8	8
Gmc(T)* x *Gmc(T)/Gmm	13	0	13
Gmc(T)* x *Gmm/Gmc(T)	6	7	13
Gmm* x *Gmm/Gmc(T)	11	0	11
Gmm* x *Gmc(T)/Gmm	2	5	7

[1] Female x 1st male/2nd male

These are quite remarkable findings and they indicate that females of some species are able to select the conspecific sperm from a mixture of two. In other cases the female can only perform this selection when conspecific male is the first mating and if the conspecific male is second then the sperm is used at random. These observations have of course some relevance to the use of hybrid sterility for tsetse control.

3. Genetic Sexing Strain Development in Medfly



Stability and productivity are probably the two most important criteria for the evaluation of genetic sexing strains (GSS). Stability has been considerably improved over the last years to a level where it becomes exceedingly difficult to determine it under normal laboratory conditions. Very large numbers of flies have to be screened over an extended period of time to obtain reliable results that allow predictions to be made on the performance of a strain under mass rearing conditions.

In addition, productivity and, associated with it, product quality, are also affected by the structure of the translocation in the GSS. Within the theoretical limits set by the presence of a translocation in the males and the biological features of the temperature sensitive lethal (tsl) used as selectable marker, strains have been identified that are better suited for mass rearing than others. The use of an inversion has added both stability in use and flexibility in construction of GSS. In fact, the introduction of an inversion has enabled one of the very first GSS to be re-evalauted. Early results indicate a much improved quality control profile.

Genetic transformation of medfly has been very successful and many transgenic lines are available marked with both green and red fluorescence (see above). The transgenic work has been carried out in close collaboration with Dr. A. M. Handler of the USDA/ARS. The lines will be used to study aspects of stability and fitness of transgenic lines. The ultimate goal is to use this technology to provide genetically marked strains and to develop molecular methods to produce sexing strains.

The mitochondrial DNA genome has been extensively used in medfly to describe population variation and to try to identify the origin of new outbreaks. A particular mtDNA marker can also be introduced into GSS so that they can be unequivocally differentiated from wild flies in the release area. This is a very valuable tool and was used very effectively in the South African and Madeiran medfly programme to clarify the identity of flies captured in a release area.

3.1 Improving Stability in Strains carrying the Translocation T(Y-5)101

During the evolution of GSS, the 1st generation of strains was based on *white pupae* (*wp*) and 2nd generation on *wp* and *tsl*. The strains also differed with respect to the location of the translocation breakpoints relative to these markers. The latter has important implications for the stability of GSS and only the 2nd generation GSS are stable enough for mass rearing. However, the isolation of an inversion on chromosome 5 enables a reassessment to be made of some of the earlier translocations.

3.1.1 Long term stability with translocation T(Y-5)101(= strain GS-1)

This translocation is an example of a 1st generation GSS. The translocation breakpoint is at 52B on the trichogen map on the left arm of chromosome 5 and is far from *wp* and *tsl* (**Figure 7**). Each time this strain was reared it showed accumulation of recombinant females to significant levels (**Figure 8**). This pattern of breakdown is typical for type-1 recombination, i.e. recombination between the translocated autosome carrying the wild type alleles of the selectable markers and the free autosome that carries the mutant alleles. Initially, type-1 recombination results in the occurrence of wild type females and mutant males at equal frequency.

Figure 7: Breakpoints, markers and recombination in medfly GSS

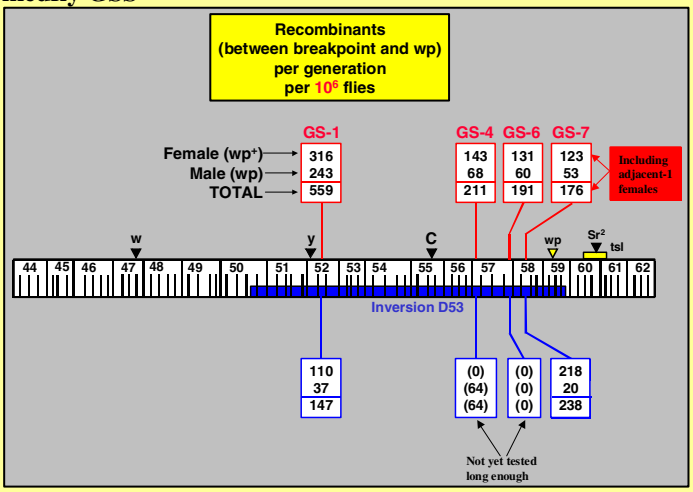
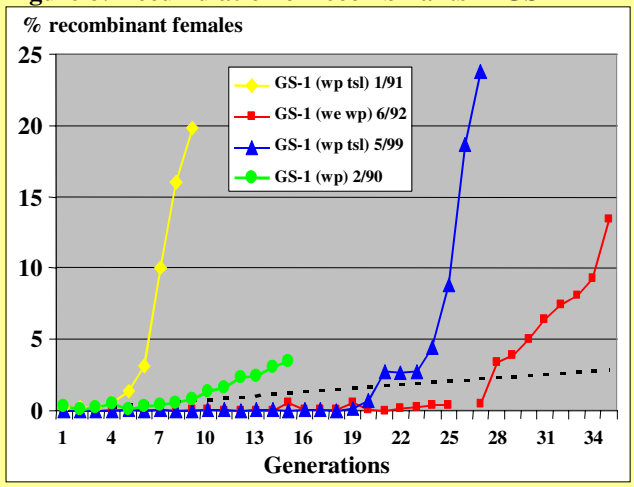


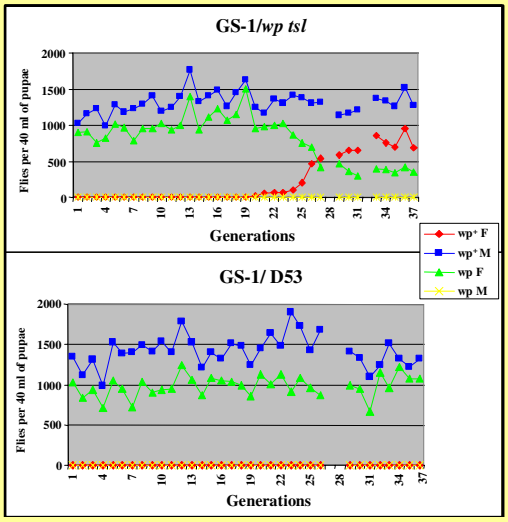
Figure 8: Accumulation of recombinants in GS-1



However, in a colony that is reared continuously, i.e. without removing these exceptional flies, the two reciprocal types of recombinants behave quite differently due to the selection pressure exerted by rearing process. The recombinant males with mutant phenotype have a selective disadvantage compared to the wild type GSS males and, therefore, their number remains low. In contrast, the recombinant females with the wild type phenotype have a selective advantage as compared to their mutant non-recombinant female siblings and they accumulate in the colony at levels above that expected on an additive basis (dotted line in **Figure 8**).

This very typical pattern was analysed over a long period of time. Each generation 34ml of unselected pupae were used to set up the next generation while the

Figure 9A: Recombinants in GS-1 and GS-1 with inversion D53.



flies from a parallel sample of 40ml were counted and checked for recombinants. **Figure 9A** shows the absolute numbers of the four principle phenotypes in this strain, i.e. non-recombinant males (wp^+) and females (wp) and the two reciprocal recombinants wp males and wp^+ females for the the two GS-1 strains. As described above, one class of reciprocal recombinants, the mutant males, does not accumulate over time. From ca generation 20 onward recombinant females accumulate at the expense of the normal, non-recombinant females. However, the overall sex ratio remains constant (**Figure 9B**) suggesting that there is some form of balancing selection operating. The accumulation of recombinant females does apparently not lead to a complete reversal of the GSS to a bisexual strain, i.e. from generation 31 onwards their numbers seems to level off and remain constant at approximately 30% (of all flies). The ratio between recombinant wp^+ females and non-recombinant wp females seems to stabilize at a value of two which could be interpreted as meaning that the wild females are approximately twice as fit as the mutant females homozygous for the markers wp and tsl (**Figure 9C**). The point at which the accumulation of wp^+ females stabilizes will probably depend on the particular rearing system being used i.e. the more stringent the rearing conditions the more the wild-type females will have the advantage.

Figure 9B: Sex ratio in GS-1 and in GS-1 with inversion D53.

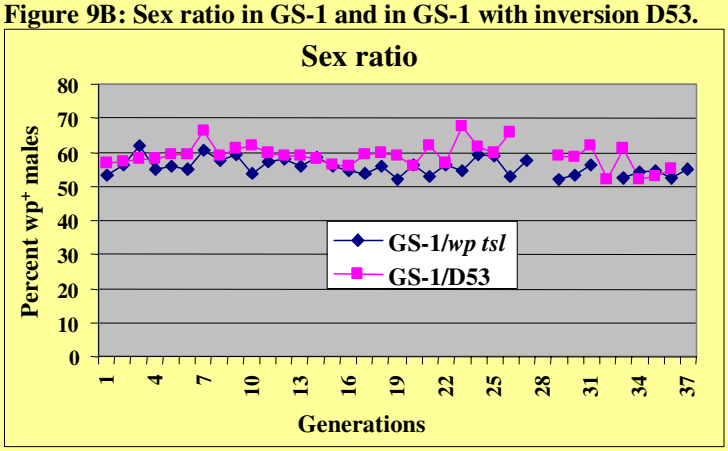
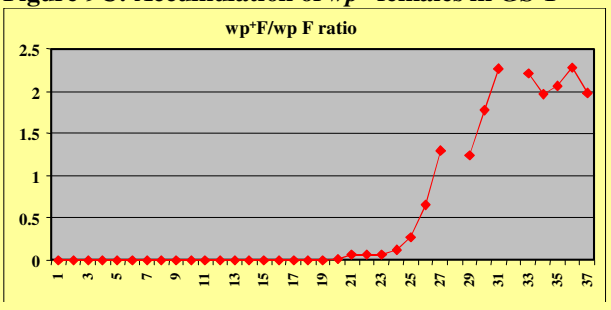


Figure 9C: Accumulation of wp^+ females in GS-1



3.1.2 Recombination between wp and the breakpoint.

To determine the actual recombination frequency between the translocation breakpoint and wp the results from the first 18 generation of strain GS-1 are used, i.e. only the generations before any clear accumulation of recombinants occurred (**Table 6**). The result is 0.056% per generation. This value can be compared with those obtained for the 2nd generation strains GS-4 (0.021%), GS-6 (0.019%) and GS-7

Table 6: Recombination frequency in different medfly GSS with and without inversion D53.

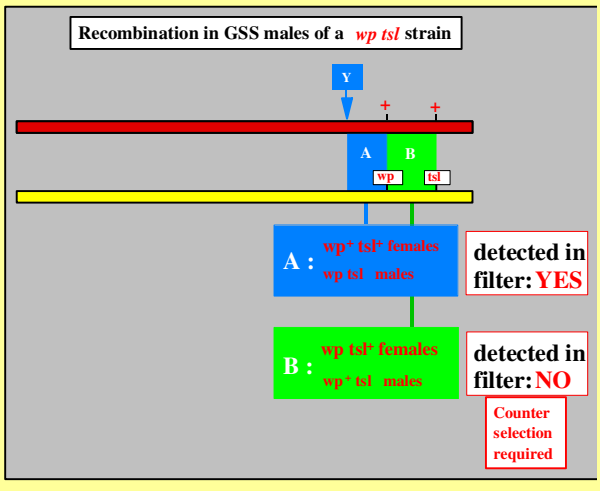
Strain	Gen.	*wp*$^+$ Females	*wp*$^+$ Males	*wp* Females	*wp* Males	% Recomb.
GS-1	37	7357	45679	29026	18	8.985
GS-1	**1-18**	**13**	**23278**	**17863**	**10**	**0.056**
GS-1/D53	**36**	**9**	**48152**	**33445**	**3**	**0.015**
VIENNA 4 A+B	26	2	23332	14448	12	0.037
4C	95	33	97326	73219	1	0.020
4E	42	5	40356	30386	6	0.016
VIENNA 4 total	**182**	**7419**	**278123**	**198387**	**50**	**0.0111**
VIENNA 4/D53	**7**	**0**	**9422**	**6251**	**1**	**0.006**
VIENNA 6 A	9	1	6897	4657	0	0.009
6 B	37	15	39576	28840	2	0.025
6 C	37	3	35745	24815	2	0.013
6 E	37	1	41501	22655	4	0.007
6 HE	36	15	37794	25667	5	0.032
VIENNA 6 total	**527**	**14873**	**727181**	**509659**	**114**	**0.019**
VIENNA 6/D53	**7**	**0**	**10252**	**5438**	**0**	**0.000**
VIENNA 7 C	6	0	5685	4527	0	0.000
7 D	56	10	59617	43950	10	0.019
7 E	67	20	73849	60057	3	0.017
VIENNA 7 total	**1190**	**29776**	**1603765**	**1133290**	**241**	**0.018**
VIENNA 7/D53 1	33	20	42297	29381	1	0.029
7/D53 13	43	16	53395	39526	2	0.019
7/D53 Mix01	8	3	8033	6258	1	0.028
VIENNA 7/D53 total	**2464**	**59591**	**3311255**	**2341745**	**486**	**0.024**

(0.018%). For GS-7 that would mean that recombination is reduced by 68% compared to GS-1. However, in this particular case the recombination frequency may even be an overestimation because the strain produces *wp*$^+$ females as a result of adjacent-1 segregation. None of 2nd generation strains showed, under the rearing regime used here, the typical "breakdown" phenomenon that was observed each time with GS-1, despite the fact that they were reared continuously for up to eight years. However, even in facilities using the Filter Rearing System (FRS) to maintain stability, the frequency of recombinants was sometimes unexpectedly high and was, at least in some cases, increasing with time. There could be several explanations for this phenomenon. Firstly, it could be due to sampling error in terms of what proportion of individuals are used to set up each generation, secondly there might be bias in which pupae are selected as founders of each generation and thirdly the quality of the rearing process may be below standard. However, recombinants can only accumulate if the cleaning process is not done properly.

3.1.3 Recombination between wp and tsl

In the practical application of 2nd generation GSS, the females are killed using the *tsl* mutation and *wp* is used only as an indirect marker to determine the presence of the *tsl*. This is made possible by the close linkage of these two markers (**see Figure 7**). However, recombination does occur between *wp* and *tsl* with the consequence that *wp tsl*$^+$ females are generated. These can only be detected with a temperature test and this is complicated and labor intensive. The standard

Figure 10: Recombination and its detection in the FRS



temperature test consists of collecting eggs over a period of 24h (3 x 100 eggs per temperature) on three different days. These eggs are directly transferred to incubators set to temperatures between 31 and 35°C (a control sample is maintained at 25°C). After incubating for 24h, the samples are returned to normal rearing at 25°C. **Table 7**

Table 7: Effect of temperature on two medfly GSS

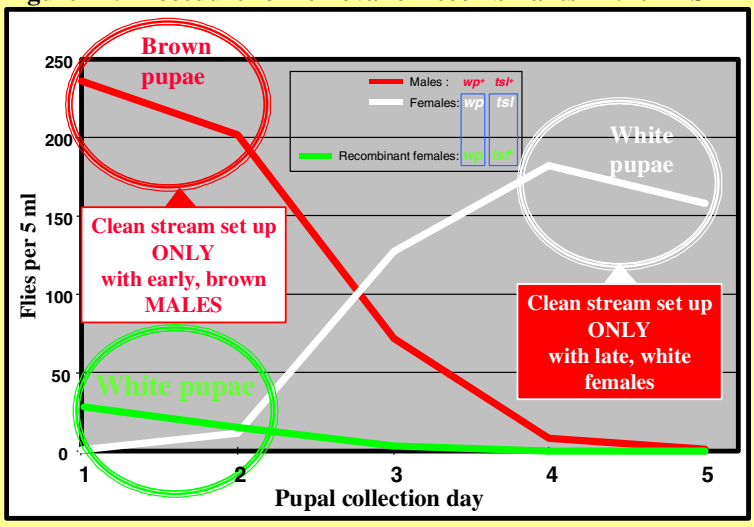
Strain	Temp (°C)	Eggs	Hatch	*wp* Pupae	*wp+* Pupae	*wp* Females	wp^+ Males	wp^+ Females
GS-1	25	900	670	260	293	220	264	1
F19	31	900	509	83	262	51	236	1
	32	900	457	36	250	9	190	1
	33	900	274	2	187	1	152	1
	34	900	226	2	158	2	128	1
	35	900	142	0	75	0	61	0
GS-1/D53	25	900	656	271	285	223	248	0
F13	31	900	643	217	294	191	263	0
	32	900	546	93	290	73	265	0
	33	900	323	10	220	6	204	0
	34	900	211	0	157	0	156	0
	35	900	156	0	115	0	105	0
Summary	25	1800	1325	531	578	443	512	
	34	1800	437	2	315	2	284	1

shows the results of such a temperature test are for GS-1 in generation F19, i.e. just before the breakdown was accelerated due to the accumulation of wp^+ females. These recombinant females are also apparent in the temperature test. However there is no obvious sign of the presence of surviving *wp* tsl^+ females. Even in the homozygous *tsl* strains it is not unusual that a few females are detected at 34°C. The test will be repeated with the current generation of GS-1 (F37).

3.1.4 Removing recombinants for the Filter Rearing System (FRS)

The occurrence of *wp* tsl^+ females is a threat to the FRS because removal of recombinants can only be done using *wp* as a marker (**Figure 10**). These particular females are undistinguishable from normal females and if they occur they have a good potential to accumulate (unnoticed) because they have lost the *tsl* mutation. The presence of such females is usually only detected in the offspring of the release colony ("male-only") by the fact that an increasing number of *wp* females survives the temperature treatment. At the level of the FRS the only known procedure to reduce the level of *wp* tsl^+ females is a counter selection strategy based on the difference in developmental speed of *tsl* and tsl^+ larvae (**Figure 11**). tsl^+ larvae (= males from brown pupae) develop faster than *tsl* larvae (= females from white pupae). As pupae are collected on a daily basis, the first collection(s) will consist primarily of wp^+ tsl^+ males whilst later collections will be primarily *wp tsl*.

Figure 11: Procedure for removal of recombinants in the FRS

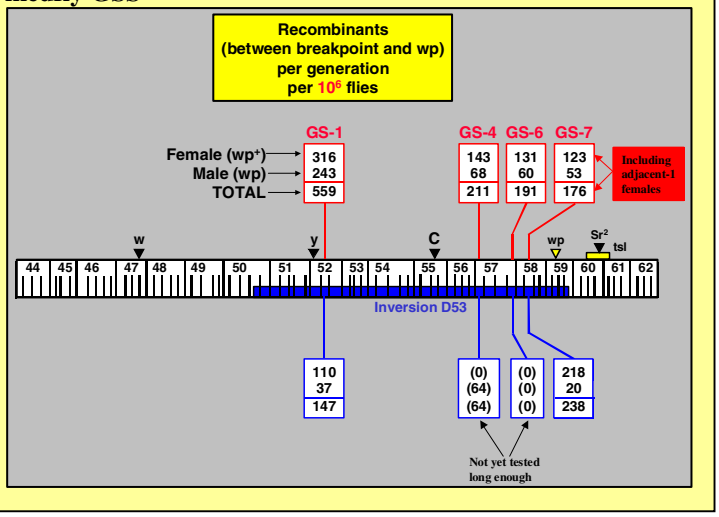


Within such a scenario, the *wp tsl*$^+$ females will be found in the earlier collection together with the males. If each generation is set up with only *wp*$^+$ males from the early collection (i.e. excluding all *wp* females) and *wp* females from the later collections, (i.e. excluding *wp tsl*$^+$ females), then this selection is very effective.

3.2 Inversions in GSS

In 2nd generation GSS, stability was increased by using Y-autosome translocations where the breakpoint is close to the selectable marker(s). However, there is a different genetic strategy that can accomplish this, i.e. the use of chromosomal inversions. Ideally, the inversion should include the translocation breakpoint and both *wp* and *tsl* but so far no such rearrangement is available. Inversion D53, (Ann. Rep. 1999) includes *wp* (**Figure 7**) and has been introduced into several GSS including GS-1 and GS-7 to measure the effect on recombination in the interval between the translocation breakpoint and *wp*. The detailed results are shown in **Table 6**. The strain GS-1/D53 was maintained in parallel to the strain without inversion for 36 generations and no breakdown was observed (**Figure 9A**). The recombination frequencies are summarized in **Figure 7**. The two recombinant classes, *wp*$^+$ females and *wp* males, are shown separately because in strain GS-7 adjacent-1 females with *wp*$^+$ phenotype occur and they cannot be easily distinguished from recombinants. If one uses only the recombinant males in a comparison of the different strains, it becomes apparent that the frequency of recombinants is reduced significantly when the inversion is included.

Figure 7: Breakpoints, markers and recombination in medfly GSS



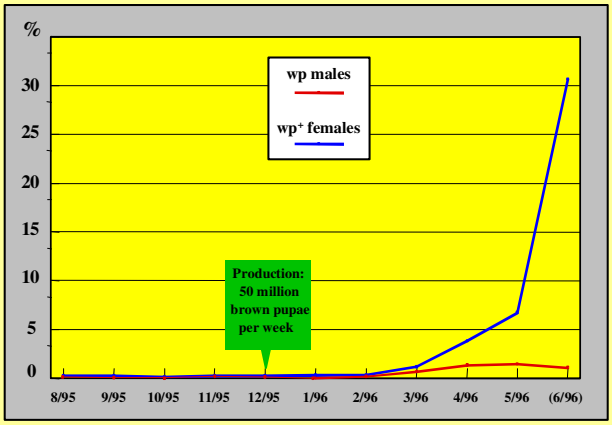
The recombination frequency in GS-1/D53 is even reduced to a level clearly below that found in the 2nd generation GSS without inversion. It has to be stressed, however, that some recombinants are still detected even though the translocation breakpoint and *wp*, are both included in the inversion. Additional experiments will be required to determine the exact nature of these flies, i.e. whether they are indeed recombinants. Experiments with GS-4 and GS-6 have been initiated to determine whether within the inversion, the distance between the translocation breakpoint and *wp* plays a role. What the effect of the inversion on recombination between *wp* and *tsl* is not yet known but preliminary results indicate that recombination is also reduced in the neighborhood of the inversion although not to the same extent as within.

3.3 Analysis of GSS from Operational Programmes

3.3.1. Samples from a GSS mass reared in Guatemala.

In 1994 the first GSS was introduced into an operational mass rearing facility in Petapa, Guatemala. The strain, VIENNA 42/Tol, was produced in Seibersdorf by introducing genetic material from Guatemala (Toliman strain) into a GSS. During the first two years of mass rearing stability of the sexing system was a problem (Ann Rep.

Figure 12: Recombinants (%) in the mass rearing of VIENNA-4/TOL



1996 and **Figure 12**). In 1994 and 1996, samples from the mass rearing colony were analyzed in Seibersdorf to determine whether the observed instability was solely caused by genetic factors or, for example, by contamination with material from a bisexual strain as was suspected for the breakdown in 1996. In addition, in 1996 a sample from the same strain that had been maintained in Seibersdorf, was analyzed (**Table 9**). In all samples from Guatemala extremely high numbers of recombinants were detected, i.e. a very high frequency of type-1 females (wp^+) and a moderate level of type-2 males (*wp*). One of the 1996 samples was obtained from temperature treated material and this showed that also the second class of type-1 females ($wp\ tsl^+$) was present (see **Table 9**). However, it is surprising that these *wp* females still seem to show the same distribution in the different pupal collections as the non-recombinant *wp tsl* females. No *wp* males were found after the temperature treatment which is consistent with their genotype (Y *wp tsl/wp tsl*).

Table 9: Detection of recombinants from different samples from Guatemala

	wp^+ Female	wp^+ Male	*wp* Female	*wp* Male
VIENNA-42/TOLIMAN (approx. F14) 5/95				
total	240	545	937	182
%	12.6	28.6	49.2	9.6
VIENNA-42/TOLIMAN 6/96				
collection	**Colony mix**			
1	163	758	39	8
2	74	877	84	4
3	78	595	149	5
4	95	488	345	20
total	410	2718	617	37
%	10.8	71.9	16.3	1.0
	Thermal treatment			
1	489	700	5	0
2	390	699	19	0
3	247	811	33	0
4	157	651	47	0
total	1283	2861	104	0
%	30.2	67.3	2.4	0.0
	Colony *tsl*			
1	515	854	12	0
2	575	799	10	0
3	352	724	30	3
4	408	656	264	5
5	292	429	636	46
6	263	308	669	32
total	2405	3770	1621	86
%	30.5	47.8	20.6	1.1

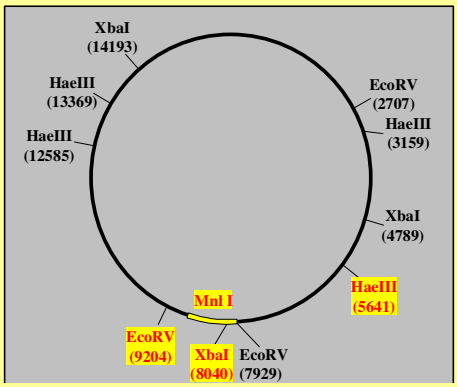
Additional crosses were done to characterize the samples further. The tests consisted of two consecutive crosses of single males with tester females carrying *white eye* (*w*) and *wp* as markers. Both samples show some quantitative but no qualitative differences. Both samples contained a surprisingly high frequency of free Y chromosomes in males emerging from brown pupae and, in general, a very high frequency of free wp^+ chromosomes. The fact that both samples are rather similar seems to exclude a contamination in 1996 although other evidence suggested that this had occurred. However, it very difficult to make this judgement with genetic analyses alone.

3.3.2 Analysis of mtDNA

An attempt was also made to identify molecular methods that would enable the GSS and the bisexual strain to be distinguished but it became apparent that the outcrossing had removed any useful differences. It was concluded that it would be

very useful if in future, GSS carried an internal tag that could be used in these scenarios. For that purpose a particular mitochondrial haplotype was introduced as marker in all new GSS (Ann. Rep. 2000). For the analysis of mitochondrial DNA (mtDNA) four different restriction enzymes are the most useful: *EcoRV*, *XbaI*, *MnlI* and *HaeIII* (**Figure 13**). The sites for *EcoRV* at position 9204, for XbaI at position 8040 and for *HaeIII* at position 5641 can either be present (=A) or absent (=B). The situation with *MnlI* is more complicated as this enzyme recognizes many sites on the mtDNA genome. One particularly variable region was chosen to compare populations. For all four enzymes the respective PCR primers encompassing the variable site are available (**Table 10**). In this PCR-RFLP analysis the fragment of the mtDNA is amplified followed by digestion with the appropriate enzyme.

Figure 13: Restriction map of medfly mtDNA



All recent GSS constructed in Seibersdorf carry the mtDNA haplotype AAAA (enzymes are in the same order as mentioned above). This haplotype was detected in the wild type strain EgII and it appears to be very useful as it represents a very rare combination, especially the type A for *HaeIII*, which until then had only been found in a population in Greece. Although this marker was originally introduced to be able to detect contamination of a GSS, the following two cases illustrate how useful this marker is for the analysis of field populations, e.g. to determine whether fertile GSS flies were accidentally released.

Table 10: mt DNA primers in medfly and the amplified fragment

Restriction Enzyme	Primer Sets	Primer Position	PCR fragment (bp)
EcoRV	5'-GACCCAGATACAGGAGCTTCT-3' (F)	8953	738
	5'-GAGTATGTGAAGGTGC-3' (R)	9691	
Xba	5'-TCCTAAACCATCTCACCC-3' (F)	7821	536
	5'-GTGGGGGAAATATTCGAGAG-3' (R)	8357	
MnlI	5'-GATATCTCCTATTGATATAT-3' (F)	7928	1048
	5'-ATAGAAGCTCCTGTATCTGG-3' (R)	8976	
HaeIII	5'-GTATGTTTAATTCGACACTT-3' (F)	5456	532
	5'-CATTCATGGTATAGTCCAAT-3' (R)	5988	

3.3.3 Analysis of flies from Madeira

Independent analyses of the wild population on Madeira, conducted before the SIT project started (before 1994, see **Table 11**), had shown that the mtDNA haplotype is BBBn or BBnB (n=not determined). This finding was remarkable as most Mediterranean samples are AAAB or AABB and it was expected that there should be a considerable influx of flies from these regions, especially from the Portugal mainland. In May 2001 new samples from Madeira and the neighboring island Porto Santo (where most of the sterile flies are released) were analyzed (B. McPheron). This analysis detected a mixture of 70% BBnn and 30% AAnn (initially only *EcoRV* and *XbaI* were analysed). One possible explanation for this result was that fertile GSS females were accidentally released. To exclude this possibility several additional samples either present in Seibersdorf or received from Madeira were analyzed. As **Table 11** shows most samples still carried the original haplotype BBBB except a Madeira wild type strain established in Seibersdorf in January 2001. Here a 50/50 mixture between BBBB and BABB was detected. The most important conclusion is

Table 11: Medfly mtDNA haplotypes in field populations and GSS strains

	EcoRV	*XbaI*	*MnlI*	*HaeIII*
EgII = GSS	A(3)	A(3)	A	A(4)
Madeira old data				
McPheron	B(2)	B(2)	B	
Seibersdorf (1994)	B(2)	B(2)		B(3)
Mediterranean	A(3)	A(3)	A or B	
Madeira new data				
McPheron (5/2001):				
Madeira and Porto Santo	B(2) 70%	B(2)	B	B(3)
	A(3) 30%	A(3)	B	B(3)
Seibersdorf				
Sample collected 1996	B(2)	B(2)	B	B(3)
Strain established 1/2001	B(2) 50%	B(2)	B	B(3)
	B(2) 50%	A(3)	B	B(3)
Samples sent 7/2001	B(2)	B(2)	B	B(3)

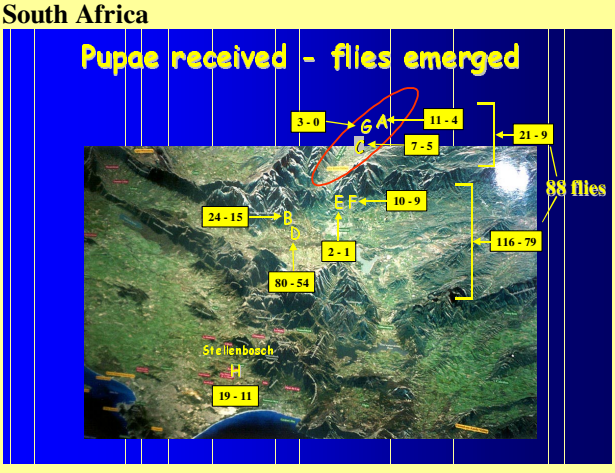
that the occurrence of the AA (*EcoRV*, *XbaI*) haplotype was not due to the release of fertile GSS females, i.e. all samples are clearly different from the GSS with its AAAA haplotype. This finding was corroborated by B. McPheron when he determined the full fingerprint of his 2001 sample to be AABB (30%) and BBBB (70%). The question whether the haplotypes BABB and AABB were only overlooked in the early analyses (potentially due to different sampling locations) or whether they represent new introductions to Madeira remains unanswered. The collaborators on Madeira are planning a more thorough sampling on Madeira itself and on Porto Santo.

3.3.4. Analysis of flies from South Africa

In December 2001, the SIT project in South Africa reported that considerable numbers of larvae were detected in grapes and other fruits in the release area and this despite the fact that only released (marked) flies were trapped i.e. no wild flies were detected. This created the suspicion that fertile GSS flies had been accidentally released. A GSS strain is being reared to produce flies for release but due to a relatively high level of recombinants, 30% sterile females are also being released. If only males are being released then even an accidental fertile release cannot increase the size of the wild population. A second important consideration is that the mtDNA is only transmitted through the female, i.e. male and female offspring of a fertile GSS female would inherit the respective mitochondrial marker.

The aim was to determine whether the mtDNA marker from the GSS could be detected in the field population. As control, 58 flies from the GSS in South Africa were analysed. The result showed that the strain was apparently contaminated with material from the previously mass reared strain. Until fall 2000 the GSS VIENNA 7/Tol from Guatemala was reared (AAAB) which was then replaced by VIENNA 7/Mix2000 from Seibersdorf (AAAA). Among the 63 flies analysed, 22% were AAAB.

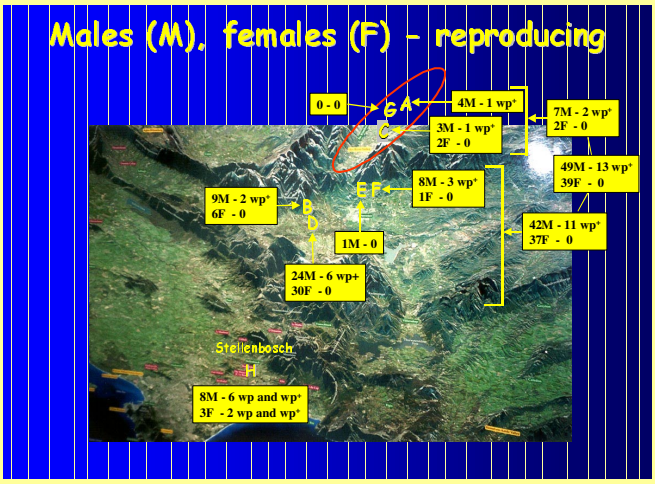
Figure 14: Emergence from pupae received from South Africa



In January 2001, several

samples from the release area (labeled A, C and G) and the buffer zone (labeled B, D, E, and F) were received (**Figure 14**). None of the 137 pupae were *wp* (the release material contained ca 7% *wp*). 88 flies emerged and were set up in cages with *w wp* test flies. **Figure 15** shows that none of the 39 females laid eggs through the egging nets which is very indicative that they were wild females. A similar argument applies for the males, i.e. only relatively few succeeded in reproducing (13 out of 49). The pupal color of the offspring of the males was brown, i.e. the parental males where homozygous for wp^+. These findings are quite different from a control sample (H) from the mass rearing facility. Here 6 out of 8 males and 2 out of 3 females reproduced and in their offspring white and brown pupae were found.

Figure 15: Reproduction in flies received from South Africa



The mtDNA analysis showed that none of the flies from the field contained either the AAAB or the AAAA haplotype (**Table 12**). All eleven flies of the control sample H were AAAA. The number of flies analyzed from the SIT area and the buffer areas are very different and it is difficult to compare haplotype frequencies. However the overall variability is relatively high compared to, e.g. Madeira and there are some very rare haplotypes, e.g. AACA. The presence of this variant demonstrates the risk of relying on only on one marker, such as *HaeIII*. On the other hand, using the full set of four enzymes, there is a very high degree of confidence that the GSS with the AAAA haplotype can be distinguishes from most, if not all, wild populations.

Table 12: mtDNA haplotypes in medfly field samples from South Africa

	MtDNA Haplotypes								#	Region
Sample	**AAAA**	**AAAB**	**BABB**	**AABB**	**BADB**	**AACA**	**BAEB**	**AADB**		
GSS	45	13							58	
A	0	0	4						4	SIT
B	0	0	7	5	2			1	15	Buffer 1
C	0	0	1	4					5	SIT
D	0	0	26	17	8	2	1		54	Buffer 1
E	0	0		1					1	Buffer 2
F	0	0	7	2					9	Buffer 2
SUM	0	0	45	29	10	2	1	1	88	TOTAL
			MtDNA haplotypes/region							
			BABB	**AABB**	**BADB**	**AACA**	**BAEB**	**AADB**		
			5	4	0	0	0	0	9	SIT
			BABB	**BABB**	**BABB**	**BABB**	**BABB**	**BABB**		
			33	22	10	2	1	1	69	Buffer 1
			BABB	**BABB**	**BABB**	**BABB**	**BABB**	**BABB**		
			7	3	0	0	0	0	10	Buffer 2
H	11	0							11	

4. Quality Control of Medfly Genetic Sexing Strains

Quality of males for SIT programmes remains of paramount concern and mechanisms to improve quality of the final product can have an immediate impact on the effectiveness of a programme. A major aspect of quality relates to male mating competitiveness. Results presented show that male medflies when exposed to certain chemicals can show a remarkable increase in this reproductive parameter. If this procedure can be efficiently incorporated into a mass rearing situation then it could have a major impact on medfly SIT.

The effect of the incorporation of any genetic marker on the quality of strain for SIT has also to be assessed, especially if that marker produces a change in the phenotype of the male. A GSS carrying a possible marker, Sergeant (Sr2) was evaluated in a field cage. No effect on several mating parameters could be seen in flies carrying this marker and in 2002 it will be introduced into the current GSS.

Within operational programmes there is considerable interest in developing a system for transporting GSS eggs from one large facility to several satellite larval rearing centers producing only males for release. This set-up has many operational advantages. Initial experiments have now shown that providing the eggs are collected over a particular time frame they can be stored in water for considerable lengths of time without losing viability. In 2002, egg shipments from Guatemala to Seibersdorf will be initiated to further develop this concept.

In support of a medfly release project in Middle East, the group initiated weekly shipments of 5 million sterile male pupae to Israel. This has required a temporary expansion of the staff and has provided a good training environment for fellows. This activity will be terminated in 2002 when other sources of pupae are identified.

A second species of fruit fly was introduced into the facility in 2001. The olive fly, Bactrocera oleae, is the major insect pest of olives across the whole range of olive production. The major challenge will be to identify an appropriate larval diet to replace the olive fruit. The very specific nature of the olive fruit will be a difficult environment to simulate although in the past considerable progress was made.

4.1 Exposure to Ginger Root Oil (GRO) Increases Mating Success of GSS Males

Tests to evaluate whether chemical preconditioning using GRO could increase mating success of GSS males were carried out in collaboration with Dr. Todd Shelly (USDA-APHIS PPQ HAWAII). Previous tests using the same compound in Hawaii and Guatemala have shown that GRO can increase mating performance of the sterile males the present experiments were carried out to confirm the results using wild flies from Madeira and Guatemala and males from the GSS strain VIENNA-7-2000.

Males from GSS strain were irradiated as pupae with 100Gy 1 day prior to eclosion and marked with a pink fluorescent dye. Wild flies were from two recently colonized strains. The Guatemala strain was reared from coffee berries (*Coffea arabica* L.) collected in Guatemala, and the other was initiated using flies reared from oranges and other hosts collected in Madeira, in both cases, pupae were shipped by air to the Seibersdorf facility. Both colonies were started with 500-1000 adults and papayas were supplied for oviposition and subsequent larval development. Adults were fed the same protein-sugar mixture as the GSS males. When used in the present study, the Guatemala and Madeira flies were 4 and 2 generations removed from the wild, respectively.

4.1.1 Mating tests

Three experiments were performed that measured the influence of GRO on the mating competitiveness of GSS males. Firstly, Guatemala males competed against GRO-exposed (treated) or non-exposed (control) GSS males for Guatemala females. Secondly, Madeira males competed against treated or control GSS males for Madeira females. Wild males were not exposed to GRO in either of these experiments. Thirdly, GRO treated, GSS males competed against non–treated GSS males for Guatemala females.

GSS males were exposed to GRO by applying 20µl of GRO to a small piece of filter paper using a microcapillary pipette. The paper was then placed on the bottom of a transparent, plastic-drinking cup (400ml volume), 25 males were placed in the cup using an aspirator, and the cup was covered with nylon screening. Exposure commenced at 1100h and continued until 1400h. GRO acted as an arrestant, and males were generally quiescent. Males did not aggregate near the filter paper and were not observed touching it. For all tests, treated males were used the day after exposure to GRO. In experiments 1 and 2, 100 GSS males (control or treated) and 100 wild males and females were used. In experiment 3, 100 treated and 100 control GSS males and 100 wild females from the Guatemala colony were used. Males were released at 0815h, and females were released 20 min later. Mating pairs were collected over the next 4h. For experiments 1 and 2, males were identified as GSS or wild using the dye. For experiment 3 (where only GSS males were used), males were marked 2 days before testing by cooling them for several minutes and placing a dot of enamel paint on the thorax. In experiment 1, wild Guatemalan males obtained significantly more matings than the control or treated GSS males (**Table 13**) but GRO exposure did improve the performance of the GSS males. In a given replicate, treated GSS males achieved significantly more matings ($T=109.0$; $P<0.05$) and a significantly higher proportion of matings (30% versus 16%, respectively; $T=114.0$; $P<0.05$) than control males. There was no difference in the number of matings obtained by wild Guatemala males ($T=92.5$; $P>0.05$) or in the total number of matings ($T=88.0$; $P>0.05$) between trials involving control versus treated GSS males.

GRO exposure had a more marked effect on the mating frequency of GSS males in experiment 2. Madeira males accounted for significantly more matings per replicate than control, GSS males, but no difference in mating frequency was detected between Madeira males and treated, GSS males **(Table 13)**. In a given replicate, treated, GSS males obtained significantly more matings (T=38.5; P<0.05) and a significantly higher proportion of matings (39% versus 16%, respectively; T=40.0; P<0.01) than control GSS males. There was no difference in the number of matings obtained by Madeira males (T=32.5; P 0.05) or in the total number of matings (T=33.0; P>0.05) between trials involving control versus treated males. In experiment 3, treated GSS males obtained significantly more matings per replicate than control GSS males **(Table 13).** Over all replicates, treated males accounted for 75% (100/134) of all matings observed.

Table 13: Field cage mating experiments with medfly males treated with GRO

Exp.	Reps	Strain	GRO Yes/No	Matings/replicate (range)	T value
1A	9	Gua.	No	39.4 (21-63)	126.0***
	9	GSS	No	8.3 (2-20)	
1B	9	Gua.	No	34.8 (19-53)	122.0***
	9	GSS	Yes	15.1 (11-27)	
2A	5	Mad.	No	32.0 (22-41)	40.5**
	5	GSS	No	6.4 (1-11)	
2B	5	Mad.	No	27.6 (16-33)	35.5N.S.
	5	GSS	Yes	19.2 (10-29)	
3	5	GSS	No	6.8 (3-10)	40.0**
	5	GSS	Yes	20.0 (18-22)	

4.1.2 Female remating

The remating frequency of Guatemalan females was compared among individuals mated first to either Guatemalan males, GSS males exposed to GRO or non-exposed, GSS males. The mated females were then given the opportunity to remate with males from the Guatemalan colony. The incidence of female remating varied independently of the strain and GRO treatment of the initial mate. The average proportion of Guatemalan females remating per replicate was 11% (range: 4-16%) for individuals first mated to Guatemalan males, 9% (range: 5–17%) for individuals first mated to non-treated GSS males, and 10% (range: 0–26%) for individuals mated first to treated, GSS males (H=0.2; df=2; P>0.05; 5 replicates were run for all 3 mating combinations).

4.1.3 Male survivorship

To determine whether exposure to GRO affected male survivorship, we placed groups of 20 treated or control males from the GSS strain in small plastic containers with ample food and water and counted survivors 5d later. All males were 3d old at the start of the experiment. GRO exposure had no apparent effect on survivorship of males from the GSS strain. On average, only 0.8 control males (range: 0–2) and 1.0 treated males (range: 0–3) died over the 5-day test period (T=215.5; P>0.05).

Exposure to GRO increased the mating success of males from a GSS strain in competition with wild males from two different source populations. Following GRO exposure, the mating frequency of GSS males increased approximately 1.9-fold (from 16% to 30% in a given replicate) and 2.4-fold (from 16% to 39% in a given replicate) in tests involving flies from Guatemala and Madeira, respectively. Although substantial, these increases were actually smaller than those observed for a mass-reared, bisexual strain in Hawaii, where the mating frequency of mass-reared males increased approximately 3-fold (from 26% to 75% in a given replicate) after GRO exposure. It appears, therefore, that although GRO exposure may consistently elevate

male mating success, the magnitude of this increase may vary among different combinations of mass-reared and wild flies. The mechanism by which GRO exposure affects male mating success is unknown, although it does not appear to reflect a simple elevation of male signaling activity. The present study also demonstrated that exposure to GRO did not adversely affect male survival and that treated and control males do not appear to differ in their ability to induce a female refractory period following mating. Based on the present results, it appears that pre-release exposure of males to GRO has the potential to increase the effectiveness of GSS in the SIT. It should be relatively easy to incorporate a treatment with GRO into the current adult male handling procedures in operational programmes both in terms of logistics and cost.

4.2 Field Cage Evaluation of *Sergeant* (Sr^2) as a Marker for Sterile Medfly Males.

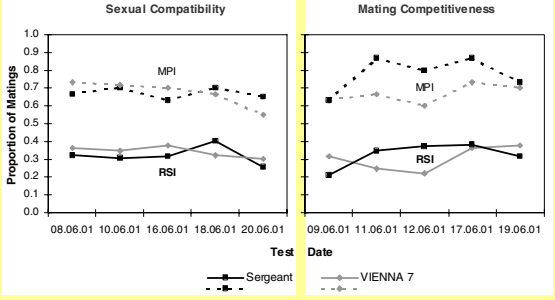
In 2000, the dominant mutation *Sergeant* (Sr^2), producing an extra, third, stripe on the abdomen of medfly, was incorporated into a GSS as a visible marker. Enormous advantages are expected to accrue to operational SIT programmes employing GSS with such a morphological marker. It could greatly facilitate and improve monitoring of released sterile males, as marking of flies with fluorescent dye could be discontinued. In order to ensure adequate quality of a GSS containing Sr^2, the strain must undergo assessment of its behavioural quality, specifically with respect to sexual compatibility of its males with wild females and their mating competitiveness against wild males. This study compared the mating performance of a novel GSS strain (Sr2) carrying the mutation Sr^2 with that of a standard mass-reared strain (VIENNA 7 Mix-2001). Mating performance was assessed in terms of (i) mating competitiveness, where tests were run with an equal number of wild females and males of any one strain (i.e. males had to compete for females); (ii) sexual compatibility, where the number of wild females equalled the total number of males of all strains released (i.e. all males had an equal chance of mating). The Guatemala strain as described above was used.

A series of two strain tests were run in which 30 GSS males (either VIENNA 7 or Sr2) and 30 Guatemalan males were released along with 60 Guatemalan females. In addition three-strain tests were conducted, where 20 males from both GSS with and without Sr2 and 20 Guatemalan were released with 60 Guatemala females. Tests lasted 4 hours and copulating pairs were counted, removed by collecting them into glass vials. Subsequently, the identity of the males was determined. For each test, the appropriate mating indices (Ann. Rep. 1998) were determined. The propensity of mating indices ranged from 0.45 to 0.78, evidence of a high general mating activity indicative of the suitability of both the flies and the environmental conditions for the tests.

4.2.1 Two Strain Tests

The mean Relative Sterility Indices (RSIs) determined for Sr2 and VIENNA 7 males in the sexual compatibility and mating competitiveness tests are shown in **Table 14** and **Figure 16** . GSS males achieved between 30 and 34% of all matings with Guatemala females in any one experiment independent of whether Sr2 was present. The two-

Figure 16: RSI and MPI for Sr2 and VIENNA 7 medfly GSS.



way ANOVA revealed no statistical difference in numbers of matings between the Sr2 and VIENNA 7 males in either of the two tests; nevertheless, Sr2 males performed slightly better in the mating competition experiment, and the reverse situation was true in the sexual compatibility test (**Table 14**). In contrast, differences in numbers of matings achieved by Guatemala *versus* GSS males were significant in both the sexual compatibility (χ^2=67.03; df=1; P<0.001) and the mating competitiveness experiments (χ^2=53.50; df=1; P<0.001), with a higher mean for Guatemala males, as expected.

4.2.2 Three-Strain Tests

In tests with Guatemala flies, Sr2 and VIENNA 7 males were equally effective in mating as wild males (**Table 14**). Nevertheless, it appears from the mean proportions of matings that Sr2 males performed slightly better with Guatemala females than did VIENNA 7 males. With respect to the sexual compatibility test, Sr2 males appeared to be significantly more successful (t=2.592; n=60; P<0.001) in mating with Guatemala females than VIENNA 7 males in a situation where each male had an equal opportunity to copulate with a female.

Table 14: Mating parameters for two medfly GSS in field cages.

	Relative Sterility Index (two strain test)		Proportion of Matings (three strain test)	
Index	**Sr2**	**VIENNA-7**	**Sr2**	**VIENNA-7**
Sexual Compatibility	0.32±0.05	0.34±0.03	0.34±0.02	0.20±0.05
Mating competitiveness	0.33±0.06	0.31±0.06	0.20±0.04	0.15±0.08

The results obtained for the behavioural tests indicate a comparable mating performance of Sr2 and VIENNA 7 when tested against wild flies. In four of the five testing protocols, the mean proportions of matings achieved by Sr2 and VIENNA 7 with Guatemala females did not deviate from each other to any statistical extent, and were within the range commonly found in mass-reared medfly strains. No adverse effects of the incorporation of the mutation (*Sr2*) into a GSS on the behavioural quality of male medfly were detected in this preliminary study. However, much more field-cage evaluation is required at other locations and the mass production characteristics need to be evaluated.

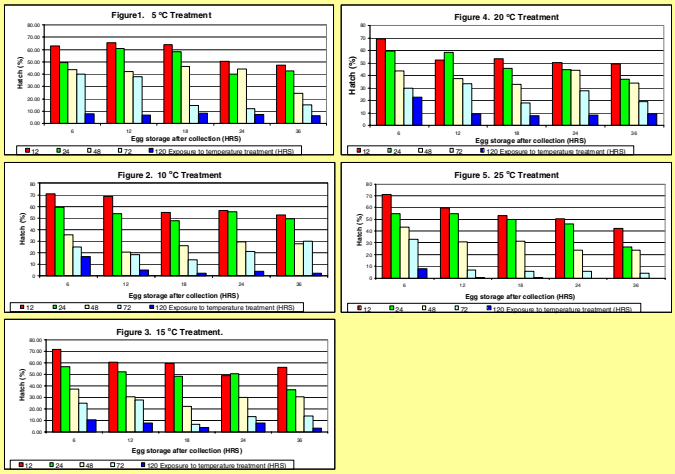
4.3 Survival of Medfly Embryos during Long-Distance Shipment in Water

In order to increase the efficiency of large operational SIT programmes and to maximise the operational use of medfly GSS, it is important to develop methods of egg shipment. This would enable a central production facility to supply eggs to satellite centers that would produce only males for irradiation and release. Initial laboratory experiments were carried out to provide protocols for testing long distance egg shipment.

4.3.1 Eggs collected 0-6 hours after oviposition

A first experiment was conducted to assess the effect on egg viability of storing eggs in water for different periods of time followed by different temperature treatments. Eggs were collected and 1ml aliquots were placed in 125, 75ml plastic flasks filled with water. 25 flasks were stored for each of the following periods: 0–6, 6–12, 12–18, 18–24 and 24–36h. Five flasks from each of the storage periods were stored at 5, 10, 15, 20 and 25ºC with 1 flask being stored for temperature treatment at each of the following times: 12, 24, 48, 72 and 120h. Immediately after treatment a sample of 1000 eggs was collected from each flask and the eggs were incubated on a

Figure 17: Effect on egg hatch of storage of eggs in water at different temperatures and for different times. Eggs collected 0-36hr after oviposition.

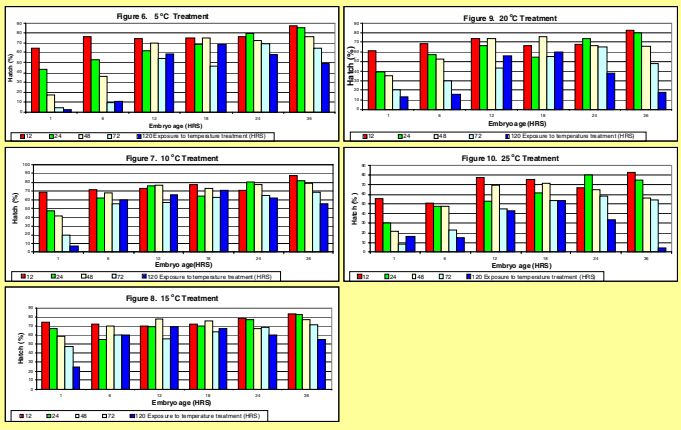


moist black filter paper at 25ºC. to determine hatch. Results shown in **Figure 17** represent the egg hatch data from the 125 treatments. It can be seen that egg hatch decreased with the length of the egg storage for all temperatures. Increasing the length of the temperature treatment also decreased the egg hatch, independent of the temperature used. The similarity in the pattern of hatch for the 5 temperatures suggests that storage of eggs in water at 24°C before treatment stops development. The viability of the embryos decreased in relationship the length of the treatment period for all temperature treatment.

4.3.2 Eggs collected 0-1 hour after oviposition

This study was carried out to assess the effect on egg viability of incubating eggs on filter paper for different periods of time followed by storage in water under different temperature treatments. Eggs were collected and 150 aliquots were transferred to moist black filter paper for incubation at 25ºC and 90%RH and 25 aliquots were incubated for each of the following periods 0-1, 1-6, 6-12, 12-18, 18-24 and 24-36h. After incubation the aliquots were transferred to flasks with water and for each incubation time 5 flasks were stored at the following temperatures, 5, 10, 15, 20 and 25ºC. From each temperature treatment, 1 flask was stored for each of the following times, 12, 24, 48, 72 and 120h. Immediately after treatment a sample of 1000 eggs was collected from each flask and the eggs were incubated on a moist black filter paper at 25ºC. to determine hatch. The data are shown in **Figure 18**. Young embryos are sensitive to storage. However, storage of embryos 12h and older is possible at different temperatures and for periods up to 72h. 24h embryos do not show a reduction in hatch when they were stored between 12h to 120h at 5,10 or 15°C. Even after 72h of storage at 20ºC no reduction of hatch was observed. 36h embryos did not show a reduction of egg hatch when stored between 12h and 72h at temperatures between 5–15°C. After 48h of storage at 20ºC no significant reduction of hatch was observed.

Figure 18: Effect on egg hatch of storage of eggs in water at different temperatures and for different times. Eggs collected 0-1hr after oviposition.



It was concluded

that for eggs collected between 0 to 6h after oviposition, the hatching rate was quite low after 12h of storage in water at any of the temperature evaluated. For eggs that had been incubated for more than 24h before storage, the hatching rate was acceptable, in spite of the several days of storage. For shipment, embryos will need to be incubated for at least 24h before being shipped in water at temperatures between 5–15ºC.

4.4 Stability of a GSS that carries a Recombination Suppressor

The optimal type of translocation in respect to mass rearing and QC would be one that generated adjacent-1 individuals that are female and homozygous for the *tsl* mutation, as these will be killed during the thermal treatment. Such a translocation T(Y-5)101 is available but as the breakpoint is in the left arm of chromosome 5 and the selectable markers are located on the right arm, this strain has an unacceptable level of recombination. As reported previously (Ann. Rep. 1999) a pericentric inversion, D53, was isolated and has now been incorporated into T(Y-5)101 to act as crossover suppressor. The stability and production and QC profiles of this strain have been evaluated. The original translocation strain is T(Y-5)101 and the strain with the inversion is called T(Y-5)101/Inv

To measure stability, both strains were mass reared at a level of $1.5x10^6$ pupae per generation under stress conditions by rearing at 26°C for the whole life cycle for 3 consecutive generations. As expected there was high female mortality during all developmental stages during the 3 generations of the high temperature rearing. At the F4 generation, the level of recombination in T(Y-5)101 had reached 21.2 % whilst in T(Y-5)101/Inv no recombinant individuals were observed (**Figure 19**). In a parallel second experiment both strains were mass reared ($3x10^6$ pupae/generation) for 22 generations under normal mass rearing conditions but with adult cages being set up with 120,000 females and 90,000 males. This would also tend to exaggerate the build up of any recombinants generated by Type-2 recombination. As expected no recombinants originating from Type-1 recombination were observed for 22 generations for T(Y-5)101/Inv. However for strain T(Y-5)101 a low frequency of recombination was observed at generation F8 but rather surprisingly the recombination value stabilised at between 0.2 to 4.0%. These preliminary data suggest that

Figure 19: Effect of an inversion on the accumulation of recombinants during rearing at 26°C for 3 generations

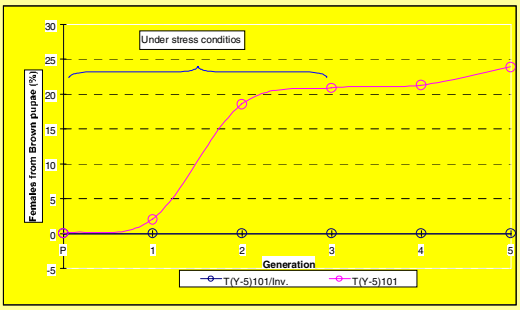
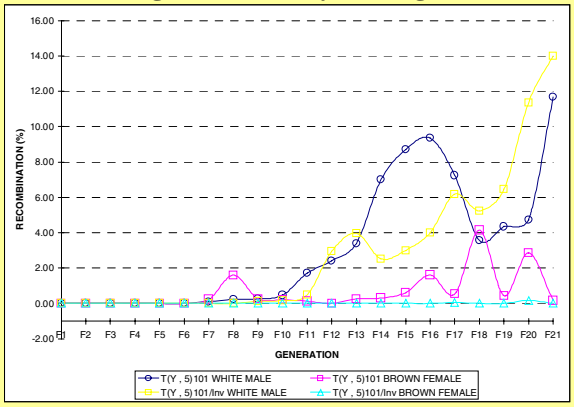


Figure 20: Effect of an inversion on the accumulation of recombinants during rearing at 20-25°C at high adult density for 22 generations.



the use of the inversion can have a significant impact on stability of a GSS in terms of Type-1 recombination. In these two experiments, the recombination was measured in terms of females emerging from brown pupae.

The second type of recombination event i.e. Type-2 will probably not be influenced by the presence of an inversion as this event is caused by recombination between the two parts of the translocated Y chromosome. This type of recombination generates males with a free Y chromosome and the accumulation of these males can be measured by monitoring the emergence of males from white pupae. In both strains the accumulation of these males was similar with more than 10% at generation 22 (**Figure 20**). Four males emerging from white pupae at generation 20 were analysed genetically and shown to be carrying a free Y chromosome. Considering the structure of the strain T(Y-5)101 it was unexpected that these males would occur so frequently.

5. Appendices

5.1 Publications

Aksoy, S., I. Maudlin, C. Dale, A.S. Robinson and S. O'Neill (2001). Future prospects for trypanosomosis control. Trends in Parasit. 17:29-35.

Franz, G. (2001) The genetic basis of SIT and all male strains. In Proceedings of the Seminar "Sterile Insect Technique as an Environmentally Friendly and Effective Insect Control System". Imprensa Regional da Madeira, E.P. pp 63-71.

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Michel, K., A. Stamenova, A.C. Pinkerton, G. Franz, A.S. Robinson, A. Gariou-Papalexiou, A. Zacharopoulou, D.A. O'Brochta and P. Atkinson. (2001). *Hermes*-mediated germ-line transformation of the Mediterranean fruit fly *Ceratitis capitata*. Insect Mol. Biol. 10:155-163

Mutika, G.N., E. Opiyo and A.S. Robinson (2001). Assessing mating performance of male *Glossina pallidipes* Austen (Diptera: Glossinidae) using a walk in cage. Bull. Ent. Res. 91:281-287.

Franz, G. Recombination between homologous autosomes in medfly *(Ceratitis capitata)* males*:* Type-1 recombination and the implication for the stability of genetic sexing strains. Genetica In press

Hendrichs, J., A.S. Robinson, J.P. Cayol and W. Enkerlin. Medfly areawide sterile insect technique programmes for prevention, suppression or eradication: The importance of mating behavior studies. Florida Ent. In press.

Mutika, G.N., E. Opiyo and A.S. Robinson. Effect of low temperature treatment on the quality of male adult *Glossina pallidipes* (Diptera: Glossinidae) in relation to the sterile insect technique. Entomol. Sci. In press.

Olet, P.A., E. Opiyo and A.S. Robinson. Sexual receptivity and age in *Glossina pallidipes* Austen (Diptera: Glossinidae). J. Appl. Entomol. In press.

Hendrichs, J. and A.S. Robinson. Sterile Insect Technique. Encyclopedia of Entomology. In press

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Todd, E.S., A.S. Robinson, C. Caceres, V. Wornoayporn, and A. Islam. Exposure to ginger root oil enhances mating success of male Mediterranean fruit flies (Diptera: Tephritidae) from a genetic sexing strain. Florida Ent. In Press.

5.2 Travel

Staff member	Destination	Period	Purpose of travel
Robinson, A.S.	Tapachula, MEX and Guatemala City, GUA	24 Jan.-12 Feb.	Review Moscamed Programme
	Rome, ITA	18-23 March	RCM D.4.20.05
	Gainesville, USA	5-12 May	Lecture at Interregional Training course
	Ceske Budejovice, CZR	28-30 June	Lecture at Biology of Disease Vectors course
	Sydney, Adelaide, AUL	6-19 July	RCM/D.4.10.15/Visit SIT programme
Caceres, C.	Tunis, TUN	25 Feb.-2 March	TC mission
	Stellenbosch, SAF	21-28 July	TC mission
	Bangkok, THA	4-11 August	TC mission
	Tunis, TUN, Athens, GRE	9-16 Sept.	TC mission/Visit Democritos Res. Centre
	Mendoza, ARG	16 Nov.-2 Dec.	RCM D.4.10.16/Fruit Fly meeting
Mutika, G.N.	Bamako, MLI and Bobo Dioulasso, BKF	9-17 Dec.	TC mission
Opiyo, E.	Pretoria, SAF	3-14 April	TC mission
	Kampala, UGA	17-24 June	TC mission
	Addis Ababa, ETH	7-15 July	RCM D4.20.06
	Ouagadougou, BKF	30 Sept.–10 Oct.	TC mission
	Kasane, BOT	10 Oct.-2 Nov.	TC mission
Wornoayporn, V.	Bangkok, THA	19 June-2 July	TC mission

5.3 Fellows

Name	Field of training	Duration (months)	Fellowship period
OLET, Ms. Pamela Akinyi	Tsetse	12.0	1999-10-05 to 2001-06-21
LU, Mr. Daguang	Medfly GS	12.0	2000-05-15 to 2001-05-14
SAHA, Mr. Ashok Kumar	Medfly	6.0	2000-09-04 to 2001-03-03
BONPLAND, Ms. G.S.	Medfly GS	1.6	2001-01-15 to 2001-02-28
RAMIREZ SANTOS, Mr. E.M.	Medfly	6.0	2001-01-15 to 2001-07-14
DRABO, Mr. Issa	Tsetse	3.0	2001-03-21 to 2001-06-20
BADO, Mr. Souleymane	Tsetse	8.0	2001-03-21 to 2001-11-20
MEDA, Ms. Aline	Tsetse	6.0	2001-06-25 to 2001-12-24
SAMOURA, Mr. Ousmane	Tsetse	3.0	2001-09-03 to 2001-12-02
SUKUPOVA, Ms. Jaroslava	Tsetse	3.0	2001-09-24 to 2001-12-23
CIAMPOR, Mr. Fedor	Tsetse	3.0	2001-09-24 to 2001-12-23
TAKAC, Mr. Peter	Tsetse	3.0	2001-09-24 to 2001-12-23
ZUBRIK, Mr. Milan	Tsetse	5.0	2001-09-24 to 2002-02-23
MAMAN, Mr. Ezequiel	Medfly	12.0	2001-10-01 to 2002-09-30
DAFFA, Mr. Curthbert Thobias	Tsetse	6.0	2001-10-01 to 2002-03-31

5.4 Insect Shipments

5.4.1. Medfly

55 shipments of fertile eggs (17l) of Vienna-7/Mix 2000 to South Africa.

6 shipments of fertile eggs (1.8l) of Vienna-7-D53/Mix 2001 to South Africa.

3 shipments of five million sterile male pupae of Vienna7-D53/Mix 2001 to Israel.

5.4.2. Tsetse

Glossina austeni **(Colony size 5,000 females)**		
Dr. Maudlin	19,350	Univ. Glasgow, UK
Dr. Gariou-Papalexiou	7,350	Univ. Patras, Greece
Dr. Aksoy	7,600	Yale Univ., USA
M. Obi	300	Swiss Tropical Inst.
Dr. Brun	300	Swiss Tropical Inst.
Dr. McCall	1800	Univ. Liverpool, UK
Dr Saccone	40	Univ. Naples, Italy
Glossina palpalis **(Colony size 5,250 females)**		
Dr. Molyneux	7,850	Univ. Salford, UK
Dr. Maudlin	7,550	Univ. Glasgow, UK
Dr. Aksoy	7,500	Yale Univ., USA
Dr. Saccone	40	Univ. Naples, Italy
Dr. Ahmed	6,200	NITR , Kaduna ,Nigeria
M. Obi	300	Swiss Tropical Inst.
Dr. Otter	690	Univ. Groningen, Holland
Dr. Brun	300	Swiss Tropical Inst.
Dr. Wetzel	50	Hanover , Germany
Glossina brevipalpis **(Colony size 3,250 females)**		
Dr. Guerin	7,050	Univ. Neuchatel, Switzerland
Dr. Aksoy	5,450	Yale Univ., USA
Glossina fuscipes **(Colony size 21,000 females)**		
Dr. Auerswald	7,500	Univ. Cape Town, South Africa
Dr. Makumi	300	KETRI, Kenya
Dr. Guerin	2,450	Univ. Neuchatel, Switzerland
M. Obi	300	Swiss Tropical Inst.
Dr. Rhoel	100	Yale Univ., USA
Dr. Otter	690	Univ. Groningen, Holland
Dr. Brun	300	Swiss Tropical Inst.
Glossina pallidipes **(Colony size 62,000 females)**		
Dr. Brun	9,350	Swiss Tropical Inst.
Dr. Gibson	9500	Univ. Bristol, UK
Dr. Gariou-Papalexiou	7,050	Univ. Patras, Greece
Dr. S. Aksoy	3,600	Yale Univ., USA
Dr. Tait	3,750	Univ. Glasgow, UK
M. Obi	300	Swiss Tropical Inst.
Dr. Saccone	40	Univ. Naples, Italy
Glossina morsitans **(Colony size 3,400 females)**		
Dr. Brun	300	Trop. Inst. Basel, Switzerland
M. Obi	600	Swiss Tropical Inst.
Dr Gariou-Papalexiou	7,250	Univ. Patras, Greece
Dr. Aksoy	7,500	Yale Univ., USA
Dr. Wetzel	50	Hanover , Germany

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