RADIATION INDUCED STERILITY TO CONTROL TSETSE FLIES

THE EFFECT OF IONISING RADIATION AND HYBRIDISATION ON TSETSE BIOLOGY AND THE USE OF THE STERILE INSECT TECHNIQUE IN INTEGRATED TSETSE CONTROL

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Marc J.B. Vreysen

PROEFSCHRIFT

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STELLINGEN

1. Het gebruik van blauwe doeken, behandeld met insecticiden is, in tegenstelling tot wat Horeth-Bontgen beweert, geen doeltreffende en economische manier om *G. austeni* te bestrijden.

   Horeth-Bontgen, D. (1992) Control of *Glossina austeni* and cattle trypanosomiasis on Unguja island by deltamethrin pour-on application to livestock and with stationary targets in cattle free zones. In: Tsetse control, diagnosis and chemotherapy using nuclear techniques, IAEA-TECDOC-634, 213-218.

2. Voorlopige resultaten van onderzoek naar het gastheerzoekgedrag van *G. austeni* zijn aanleiding om meer aandacht te besteden aan de invloed van geur op gastheerlocatie.

   Madubunyi, L.C. (1990) Ecological studies of *Glossina austeni* Newstead at Jozani forest, Unguja island, Zanzibar. Insect Science and its Application 11, 309-313 en 'Dit proefschrift'.

3. Toepassing van de 'pour-on' technologie op wilde dieren schijnt een vergezochte en on-orthodoxe manier van tsetsebestrijding te zijn, maar het zou vruchtbare resultaten kunnen opleveren voor moeilijk te bestrijden vliegen zoals *G. austeni*.

   Thomson, M.C. (1987) The effect on tsetse flies (*Glossina* spp.) of deltamethrin applied to cattle either as spray or incorporated into eartags. Tropical Pest Management 33, 329-325.

   Bauer, B., Petrich-Bauer, J. Pohlit, H. and Kabore, I. (1988) Effects of flumethrin pour-on against *Glossina palpalis gambiensis* (Diptera: Glossinidae). Tropical Medicine and Parasitology 39, 151-152.

4. Het vervangen van vers bloed door een kunstmatig dieet om de in-vitro techniek voor het kweken van grote aantallen tsetsevliegen in de nabije toekomst in Afrika te verbeteren, is geen bruikbare optie.

   Kabayo, J.P., Taher, M. & Van der Vloedt, A.M.V. (1985) Development of a synthetic diet for *Glossina* (Diptera: Glossinidae). Bulletin of Entomological Research, 75, 635-640.

5. Een vergaande automatisering van het kweken en distribueren van tsetsevliegen is een conditio sine qua non om de steriele insekten techniek voor het op grote schaal bestrijden van tsetsevliegen betaalbaar en technisch mogelijk te maken.

   'Dit proefschrift'.

6. De opinie dat tsetse uitroeiingsprogramma's het bestaan van de nationale parken in Afrika bedreigen getuigt van kortzichtigheid.

   Tarangire National Park. Published by the Tanzanian National Parks in association with the African Wildlife Foundation.

7. Tsetse bestrijdingsprogramma's die steunen op het gebruik van vallen en 'targets' in combinatie met deelname van de lokale bevolking laten, in tegenstelling tot wat beweerd wordt door Dransfield e.a., vaak te wensen over.

   Dransfield, R.D., Brightwell, R., Kyorku, C. & Williams B. (1990) Control of tsetse fly (Diptera - Glossinidae) populations using traps at Nguruman, south-west Kenya. Bulletin of Entomological Research, 80, 265-276.

8. Privatisering van de veterinaire diensten in Afrika schijnt de enige zinvolle oplossing te zijn om weer tot een efficiënte dienstverlening te komen.

9. Het ontbreken van wetenschappelijke eerlijkheid in ontwikkelingsprogramma's die steunen op technische 'know-how' kan leiden tot het mislukken van de programma's.

10. De val van het communisme en de invoering van een vrije markteconomie in de voormalige Sovjet Unie vormen een enorme bedreiging voor flora en fauna in de ongerepte natuurgebieden van Siberia.

11. De opinie dat het actief beoefenen van sport een ideaal middel is tot vermagering berust op een misvatting.

Stellingen behorende bij het proefschrift "Radiation induced sterility to control tsetse flies" door Marc J.B. Vreysen.

Wageningen, dinsdag 19 december, 1995

To Carine, who went the extra mile with me

Table of contents

Foreword

The majority of the work presented here was conducted during my assignment as FAO Associate Professional Officer at the Entomology Unit of the IAEA's laboratory in Seibersdorf, Austria. The remaining part of the research was carried out during my assignment as FAO/IAEA expert on Unguja, Zanzibar. Many people have made valuable contributions to this work and I would like to express my sincere thanks to them all. However, I have to single out Prof. Dr. J.C. van Lenteren and Dr. ir. W. Takken, for accepting the task, despite their heavy schedule, of being promoter and co-promoter of this work.

Many thanks are due to my superiors of the Joint FAO/IAEA Division, International Atomic Energy Agency for their interest in the work, their continuous support, their valuable discussions and assistance in the design of some of the experiments: Dr. B. Sigurbjörnsönn, Former Director of the Joint FAO/IAEA Division, IAEA , Dr. D. Lindquist, former Head Insect and Pest Control Section, Dr. R. Gingrich, Former Head Entomology Unit, Seibersdorf Laboratory and Drs. J. Kabayo and U. Feldmann, both former Team Leaders of the Tsetse Unit, Seibersdorf Laboratory.

I am specially indebted to the late Dr. André Van der Vloedt, Senior Tsetse officer, Insect and Pest Control Section, who introduced me to the tsetse fly and who was my direct supervisor of the work conducted at the Seibersdorf Laboratory. His vast knowledge of the tsetse and trypanosomiasis problem, his dynamic personality, his severe but always positive and constructive criticism, his unsaturable energy, his infectious enthusiasm and creative spirit have been extremely valuable during my stay in Vienna. Likewise, his encouragement during my first years in Zanzibar was very important. A great void has been created since his passing away on 31 December 1991.

I would also like to thank other IAEA staff members for their assistance: Dr. P. Kerremans, Genetic Sexing Medfly, Dr. A. Economopoulos, Medfly Mass Rearing, Dr. H. Barnor and Mr. M. Taher (Tsetse Group). Many thanks are due to the laboratory technicians of the tsetse group: Mr. R. Acs, Mr. F. Ivanschitz, Mr. F. Fröhlich, Mrs. M. Kulzer, Mrs. M. Niedrist, Mr. R. Boigner, Mrs. O. Ibanschitz and Mrs. G. Germeshausen, for their assistance in maintaining and feeding the experimental flies, for their comradeship, and especially for creating a memorable, friendly working atmosphere.

During my 5-year stay on Zanzibar, the support of Mr. M.K. Gao and Dr. A. Msangi, former and current director of the TTRI, Mr. K. Biwi, former director of the Livestock Institute and current Deputy Principal Secretary, Ministry of Agriculture and Dr. I. Shambwana, Assistant Commissioner for Livestock Development, is greatly appreciated. The laboratory experiments carried out at the TTRI would not have been possible without the help of Mr. O. Chalo and Mr. G. Mashenga, senior lab technicians, who delivered valuable assistance. Special thanks are due to Mrs. F. Mramba, Scientific Officer, who assisted me when I arrived in Tanzania. The work on Unguja would not have been possible without the support of the entire DLDZ staff especially Mr. I. Khamis, Mr. S. Khalfan, Mr. A.O. Faki, Mr. A. Abdallah, Mr. M.I. Mussa, and Bibi Masha and Bibi Amina. A special word of thanks is due for the many villagers living around the Jozani forest. Many were engaged as fly collectors, and their dedication to the work has been very important.

Last but not least, I am greatly indebted to my wife, Carine and son Tim, for their unmeasurable support during the research and for coping without complaints with the many lonely hours. Many thanks to my wife for editing the numerous drafts of the manuscripts.

Samenvatting

Genetische bestrijding van tsetse vliegen is mogelijk door ofwel (a) mannelijke vliegen met dominante lethale mutaties in de zaadcellen, veroorzaakt door bestraling van de poppen of adulte vliegen, vrij te laten in het te behandelen gebied, ofwel (b) het aanwenden van hybride steriliteit door het loslaten van verwante tsetse-soorten of ondersoorten. In dit proefschrift wordt de invloed van bestraling en hybridisatie op de voortplantingsbiologie en overleving van verschillende soorten van tsetse vliegen beschreven. Het belicht eveneens enkele aspecten van het vrijlaten van steriele vliegen in het veld.

De belangrijkste resultaten kunnen als volgt worden samengevat:

(1) In het eerste gedeelte van het proefschrift wordt de invloed van een toenemende dosis gamma-bestraling op de voortplanting en levensduur van drie economisch belangrijke soorten van tsetse vliegen beschreven. Een dosis van 50 Gy, 80 Gy en 120 Gy veroorzaakte 95% dominante lethale mutaties in de zaadcellen van respectievelijk *G. brevipalpis*, *G. fuscipes fuscipes* en *G. tachinoides*. De bestraling had geen negatieve invloed op het inseminatievermogen van de mannelijke vliegen en op de beweeglijkheid van de zaadcellen (hoofdstuk 2). De gemiddelde levensduur van de bestraalde steriele mannetjes was beduidend korter dan van een controle groep, met uitzondering van mannetjes van de soort *G. brevipalpis*, die na behandeling met een dosis van 10 - 40 Gy significant langer leefden. Het hoger aantal dominante lethale mutaties in de zaadcellen van de 3 soorten was te merken aan het toenemend aantal vrouwtjes dat na paring onevenwichtigheden vertoonden in uterusinhoud en ovariële ontwikkeling. Dit onderzoek toonde aan dat verschillende soorten van tsetse vliegen in verschillende mate gevoelig zijn voor bestraling.

(2) De invloed van ioniserende bestraling op *G. tachinoides* poppen wordt behandeld in de hoofdstukken 3 tot 5. Bestraling in lucht van 25-28 dagen oude poppen met een dosis van 10 tot 120 Gy, had geen negatieve invloed op hun ontwikkeling (Hoofdstuk 3). Op een leeftijd van 15 tot 20 dagen waren mannelijke poppen gevoeliger voor bestraling dan vrouwelijke. Behandeling van 25-28 dagen oude poppen veroorzaakte bovendien een hogere graad van steriliteit dan

behandeling van volwassen vliegen met dezelfde dosis. Bestraling in lucht kan worden uitgevoerd op *G. tachinoides* poppen vanaf een leeftijd van 20 dagen, om steriele mannetjes te verkrijgen met een bepaalde levensverwachting, te weten een residuele vruchtbaarheid van 5% of minder en een levensverwachting van 20 dagen of meer. Bestraling van jongere poppen resulteerde in een hogere mortaliteit van de poppen of een verminderde inseminatie-capaciteit van volwassen mannetjes.
Behandelen van poppen in de middenfase van hun ontwikkeling bleek mogelijk indien de bestraling plaats vond in een stikstof atmosfeer en ze toegediend werd in 2 doses; 10 en 30 Gy met een interval van 2 dagen of 10 en 50 - 70 Gy met een interval van 5 dagen (Hoofdstuk 4). Ten einde de periode van bestraling tot ontpopping te verlengen, werd een periode van koeling toegepast gedurende de popontwikkeling. De resultaten waren echter ontgoochelend. Slechts mannelijke vliegen, die als 5 dagen oude poppen geincubeerd waren bij 15 °C gedurende 9 dagen, en bovendien bestraald werden met 10 Gy in lucht, bleken steriele vliegen met voldoende levensverwachting op te leveren. De conclusie is dat tsetse poppen (*G. tachinoides*), bestraald tijdens de middenfase van hun ontwikkeling, steriele mannetjes van goede kwaliteit kunnen leveren.

(3) Vrouwelijke vliegen, behandeld met gamma-stralen kunnen vrijgelaten worden voor entomologische evaluatie campagnes (bv. in gebieden met een lage populatiedichtheid), of om uitroeiing van een populatie te bevestigen. Een lage dosis gamma-stralen (40 - 60 Gy), toegediend aan *G. austeni* poppen in de laatste fase van hun ontwikkeling of aan volwassen vliegen (2 - 9 dagen oude *G. austeni* en *G. tachinoides*) konden volledig gesteriliseerd worden zonder het paringsgedrag te veranderen (Hoofdstukken 6 en 7). Bestraalde vrouwelijke vliegen bleven tot 15 dagen na ontpopping paringsbereid (84% en 32-42% paring bij respectievelijk *G. austeni* en *G. tachinoides*). De ontwikkeling van de follikels in de ovariolen was afhankelijk van het tijdstip van bestraling. Vrouwelijke, ongepaarde *G. austeni* vliegen, al dan niet bestraald, vertoonden tot op de leeftijd van 15 dagen paringsbereidheid met niet bestraalde mannetjes (vastgesteld tijdens proeven in laboratoriumkooien). Dit gold ook voor *G. tachinoides* tot op de ouderdom van 12 dagen. Bestraalde en niet -bestraalde *G. austeni*-vliegen en bestraalde *G.tachinoides*-vliegen vertoonden een hoge graad van meervoudig

paren. Verhoging van de totale bestralingsdosis en het bestralen van oudere vliegen verminderde het meervoudig paringsgedrag.

(4) Het tweede gedeelte van het proefschrift handelt over het kruisen van verwante ondersoorten. *G. palpalis palpalis (Gpp) en G. palpalis gambiensis (Gpg)* (Hoofdstuk 8) en de soorten *Gpp* en *G. fuscipes fuscipes (Gff)* (Hoofdstuk 10) kruisten zich gemakkelijk in het laboratorium en niets wees op selectieve paring. Het bestralen van *Gpp-* en *Gpg* -mannetjes met een dosis van 120 Gy veranderde het gedrag niet gedurende het paren met vrouwtjes van de andere ondersoort en alle bevruchte vrouwtjes waren steriel. Proeven waarbij niet bestraalde, ongepaarde *Gpp*-vrouwtjes samengebracht werden met niet bestraalde *Gpp*-mannetjes en bestraalde *Gpg*-mannetjes, wezen uit dat het aantal nakomelingen van de *Gpp*-vrouwtjes proportioneel afnam met een toenemend aantal steriele *Gpg* mannetjes. Dit niet selectieve paringsgedrag tussen soorten of ondersoorten van de *palpalis* groep kan aangewend worden in uitroeiïngscampagnes door bestraalde steriele mannetjes van de ene soort vrij te laten in het gebied van de andere (Hoofdstuk 8)
Beide ondersoorten konden worden onderscheiden op basis van de breedte van de terminale dilatatie van de inferieure claspers van mannelijke *Gpp-* en *Gpg* -vliegen (hoofdstuk 10). Mannelijke hybriden vertoonden tussenliggende waarden maar de gemiddelde grootte van de terminale dilatatie werd beinvloed door de maternale afkomst. Vrouwelijke *Gpp-* en *Gpg*-vliegen konden worden gescheiden (met een geringe overlapping (7%)) op basis van morfometrische waarden van de lengte en breedte van de dorsale en anale platen. Vrouwelijke hybriden vertoonden tussenliggende waarden en een overlapping van 18%.
Teneinde een *Gpp*-paring van een *Gff*-paring te onderscheiden, wordt een methode beschreven gebaseerd op de parameters lengte, breedte en de afstand tussen de middelpunten van de paringslittekens van de 2 soorten (Hoofdstuk 11).

(5) In het derde gedeelte worden enige aspecten van het operationeel SIT programma tegen *G. austeni*-vliegen op Unguja (Zanzibar) belicht. Speciale aandacht wordt besteed aan evaluatiemethoden en aan kwaliteitscontrole van de steriele mannetjes.
Panelen bezet met een kleefstof worden gebruikt als alternatief voor normale tsetsevallen om *G. austeni*-populaties te evalueren. De doeltreffendheid van de verschillende soorten panelen om *G. austeni* te vangen op Unguja werd nagegaan (Hoofdstuk 13). De

verschillende vorm- en kleur-combinaties van een bepaald, met kleefstof bestreken paneel (monopaneel), hadden geen invloed op het aantal gevangen vliegen en op hun geslachtsverhouding. Met "voetpanelen" (voorzien van vierkante platen aan de onderzijde), blauw gekleurd aan de ene zijde en wit aan de andere, werden signifikant meer vliegen gevangen dan met panelen in andere kleurcombinaties. Deze "voetpanelen" vingen echter beduidend minder vrouwtjes. De grootte van de vangst en de geslachts-verhouding werden in grote mate beinvloed door het soort van kleefstof. Significante associaties werden gevonden tussen paneelkleur/kleefstof, leeftijdssamenstelling en moment van voortplantingscyclus van de vouwelijke vliegen.

(6) De kwaliteit van bestraalde mannelijke *G. austeni* vliegen werd onderzocht zowel in het laboratorium na behandeling, gedurende transport met een klein vliegtuig van het kweekcentrum naar Unguja , als na vrijlating in het veld (Hoofdstuk 14). Van de ruim 800.000 vervoerde steriele mannetjes werden er meer dan 90% in het veld vrijgelaten. De gemiddelde levensduur van de vrijgelaten vliegen, berekend aan de hand van het aantal teruggevangen vliegen, lag tussen de 5 à 7 dagen, met een dagelijkse mortaliteit van 10 tot 14%. In het veld werd de paringsfrequentie bepaald aan de hand van metingen van de breedte van het apicale einde van de bijklieren van mannelijke vliegen .

(7) De invloed van doeken behandeld met insecticiden gevolgd door het vrijlaten van steriele mannetjes, op de *G. austeni* vliegenpopulatie op het eiland Unguja, wordt besproken in het laatste hoofdstuk (15). Het plaatsen van 30 to 70 blauwe doeken per km^2, verminderde na een periode van 16 maanden de vliegenpopulatie tot 3 à 36% ten opzichte van de oorspronkelijke populatiedichtheid. Ook tijdens het vrijlaten van steriele mannetjes bij een verhouding van 3 à 5 steriele mannetjes per fertiel mannetje, bleef de vliegenpopulatie zeer laag. Zestien tot 27% van de wilde vrouwelijke vliegen bleek steriel als gevolg van een paring met een bestraald mannetje.

Summary

The induction of dominant lethal mutations by exposing tsetse flies as pupae or adult insects to ionising radiation and the use of hybrid sterility resulting from crosses of closely related tsetse species or subspecies, are potential methods of genetic control of tsetse flies. In this thesis the effects of radiation and hybridisation on the reproductive biology and fitness of several species of tsetse flies has been examined. In addition, aspects of field releases of sterile insects have also been examined. The major findings can be summarised as follows:

(1) In the first part of the work presented here, the effects on reproduction and survival of three important economic species following exposure to increasing doses of gamma radiation of adult male flies were investigated. A dose of 50 Gy, 80 Gy and 120 Gy induced 95% dominant lethal mutations in the sperm of *G. brevipalpis, G. f. fuscipes* and *G. tachinoides* respectively, without any negative effects on the insemination potential of the male flies and sperm motility (chapter 2). The average longevity of the sterilised males was significantly reduced as compared to untreated control males, except for *G. brevipalpis.* On the contrary, male *G. brevipalpis,* treated with doses between 10 and 40 Gy showed a significant radiation induced increase in average life span. The higher proportion of dominant lethals in the sperm of the three species was reflected in increasing proportions of their female mates showing imbalances between uterine content and ovarian development. It can be concluded that different species of tsetse display different levels of radio-sensitivity,

(2) A series of papers (chapter 3-5) reports on the effects of ionising radiation on *G. tachinoides* pupae. Radiation treatments in air up to 120 Gy, administered to 25-28 day old pupae, had no negative effect on pupal development (chapter 3). At younger stages (15 - 20 day), male pupae were more susceptible to radiation treatments than female pupae. Treatment of 25-28 day old pupae resulted in higher levels of sterility compared to adult flies, treated with the same dose. Twenty day-old *G. tachinoides* pupae are the youngest stages which can be treated in air to obtain sterile males with adequate survival i.e. a residual fertility of $\leq$ 5% and average longevity $\geq$ 20 days. Treatment of younger pupae resulted in high pupal death, increased mortality during the first week of their

adult life or reduced male insemination capacity. Treating pupae in the mid pupal phase was feasible provided the radiation treatment was administered in a nitrogen atmosphere and in doses split into 2 fractions (total dose of 40 Gy separated by 1-2 days (day 15-17) or total dose of 60-80 Gy separated by 5 days (day 15-20)) (chapter 4). Combining an irradiation treatment with a cooling period, with the aim to further increase the time frame between the radiation treatment and eclosion, was limited. Only male flies, incubated as 5 day old pupae at 15°C for 9 days and irradiated with 10 Gy in air on day 20 PL could be rendered sterile with adequate survival (chapter 5). These experiments demonstrated that good quality, sterile males can be obtained by irradiation of tsetse pupae (*G. tachinoides*) in the mid-pupal phase,

(3) Gamma sterilised female flies can be deployed for entomological monitoring in areas with low native fly densities or to confirm the status of eradication after termination of control operations. Low doses of gamma radiation (40-60 Gy) administered to late stage pupae (*G. austeni*) or to 2 - 9 day old adults (*G. austeni* and *G. tachinoides*) induced complete sterility without affecting mating behaviour (chapter 6-7). Treated female flies remained highly receptive to mating up to 15 days following emergence (84% and 33-42% mating response for *G. austeni* and *G. tachinoides* respectively). The dynamics of the follicle development were influenced by the timing of the radiation treatment. Virgin untreated and treated female *G. austeni* were equally receptive (up to the age of 15 days) to mating with untreated males in laboratory cage tests. The same observation was made with *G. tachinoides* up to the age of 12 days. A high degree of multiple mating was observed for both untreated and treated *G. austeni* and treated *G. tachinoides.* The rate of multiple mating decreased with increasing radiation doses and with treatments administered later in the female life.

(4) The second part of the work presented, concentrated on cross-breeding of closely related *palpalis* species.
The closely related subspecies *G. palpalis palpalis (Gpp)* and *G. palpalis gambiensis (Gpg)* (chapter 8) and the species *G. palpalis palpalis* and *G. fuscipes fuscipes (Gff)* (chapter 10) hybridised readily in the laboratory. No indicating of selective mating was observed during laboratory cage tests.

The mating behaviour of *Gpp* and *Gpg* in intersubspecific matings with gamma treated males (120 Gy) was not altered and complete sterility was induced in all inseminated female mates. In ratio tests with untreated virgin *Gpp* females, untreated *Gpp* males and increasing ratios of gamma sterilised *Gpg* males, a gradual decrease in production of viable offspring was observed. The use of the high hybridisation capacity of the two subspecies in combination with radiation induced sterility is proposed for genetic control (chapter 8).

The two subspecies *Gpp* and *Gpg* could be separated morphometrically based upon the width of the terminal dilatations of the inferior claspers (chapter 10). Male hybrids had intermediate values but the average size of the head of the parameres was influenced by maternal descendance. Females *Gpp* and *Gpg* could likewise be separated with minimal overlap (7%) based upon the length and width of the dorsal and anal genital plates. Female hybrids had intermediate values and characteristics overlapped for 18%.

A method was described to discriminate between matings of *Gpp* and *Gff* based upon the parameters length, width and the distance between the centres of the mating scars of the two species (chapter 11).

(5) In the third part, some aspects of the ongoing SIT programme on Unguja island against *G. austeni* are highlighted with special reference to monitoring and quality control procedures of the sterile males.

Panels, made sticky with a non-setting adhesive, are used as an alternative for monitoring populations of *G. austeni*. The efficiency of the various models for catching *G. austeni* on Zanzibar was tested (chapter 13). Panel shape and different colour combinations of one type of sticky panel (monopanel) had no effect on catch rate and sex ratio. Legpanels, coloured blue on one side and white on the other, caught significantly more flies than all other colour combinations but female flies were significantly undersampled. The type of non-setting adhesive used for rendering the surface of the panels sticky, influenced significantly the catch rate and female ratio. Significant associations were found between panel colour/sticky material, age composition and reproductive status of the female flies in the sample.

(6) Quality of gamma sterilised male *G. austeni* was assessed in the laboratory (after handling), during transport by light aircraft (from the mass rearing facility to the island of Unguja, Zanzibar) and after release in the field (chapter 14). More than 800,000 sterile males were transported of which more than 90% were actually released. Average survival of released flies, based upon recapture data, fluctuated between 5-7 days with a daily mortality estimated at 10-14%. Mating frequency of recaptured sterile males was estimated based upon the measurements of the width of the apical body of the accessory glands.

(7) the impact of Insecticide Impregnated Screens followed by the release of sterile males on the *G. austeni* population in the Jozani forest on Unguja island was assesssed. Blue cloth screens, impregnated with insecticides and deployed at densities of 30-70 per km^2, reduced the original *G. austeni* fly population density to 3 - 36% of its initial level after 16 months. The native fly population remained very low following releases of sterile males. An average ratio of 3-5 sterile males for 1 fertile male was obtained. With these ratios, a level of 16 - 27% induced sterility was observed in the wild female fly population.

Chapter 1

INTRODUCTION

' In modern entomology, the issue is not to control insects but how to control them'
J.W. SNOW (1988)

Contents

1. Tsetse flies and trypanosomiasis in Africa

There are 23 living tsetse fly species and 8 subspecies known to science (Moloo, 1993). Together with the four fossil species, located in the Florissant beds of Colorado, they are placed within the genus *Glossina* (Buxton, 1955). They are the vectors of a debilitating and commonly fatal disease of domestic livestock (animal trypanosomiasis (nagana)) and humans (sleeping sickness). Tsetse flies occur in 38 African countries and their distribution covers approximately 10 million km^2. The disease is considered the most important limiting factor for the development of the livestock sector in Africa. With more than 50 million cattle at risk of becoming infected with the disease, the direct losses in animal production and reproduction due to morbidity, mortality, infertility and the operation of tsetse/ trypanosomiasis control operations is estimated at > US$ 500 million annually (ILRAD, 1993). Moreover, according to figures provided by the WHO, 20,000 new cases of human sleeping sickness are reported every year.

The disease is caused by various species of *Trypanosoma,* parasitic protozoa found in the blood and tissues of a wide variety of vertebrate hosts. The link between tsetse flies, the trypanosomes and the disease was only discovered in 1895 (Bruce, 1895). Tsetse transmitted trypanosomes are elongated protozoans with a single nucleus. A flagellum, attached to the body by means of an undulating membrane, provides the necessary propulsion. All tsetse transmitted trypanosomes are classified in the Section Salivaria, Family of Trypanosomatidae which contains four Subgenera: Dutonnella, Nannomonas, Trypanozoon and Pycnomonas (Table 1). The various species can be distinguished by morphological characters, epidemiological differences and the site of development in the tsetse fly. When the trypanosomes are ingested by the fly together with the mammalian host's blood, they undergo a cycle of development within the insect. Trypomastigote forms, found only in the gut of the insects, migrate to the mouth parts or salivary glands where they are transformed into epimastigote forms and finally into metacyclic forms which are infective to the mammalian host. The duration of the cycle depends on the trypanosome species and the temperature.

2. Tsetse species and their distribution with special reference to those used in this study

The genus *Glossina* is divided into 3 distinct groups based upon the

Table 1. Tsetse transmitted trypanosomes and their site of development in the tsetse fly (after Hoare, 1970 & Jordan, 1986)

Subgenus	Species	Site of development in tsetse fly			Importance
		Proboscis	Midgut	Salivary glands	
Dutonella	*T. vivax*	T., E.,Mt.	-	-	Disease of cattle
	T. uniforme	T., E.,Mt.	-	-	Mild disease
Nannomonas	*T. congolense*	T., E.,Mt.	T.	-	Major disease of cattle
	T.simiae	T., E.,Mt.	T.	-	Acute disease of domestic pigs
Trypanozoon	*T. brucei brucei*	-	T.	T., E.,Mt.	Chronic disease of cattle/pigs Acute disease of dogs/horses
	T. brucei rhodesiense	-	T.	T., E.,Mt.	Acute human sleeping sickness
	T. brucei gambiense	-	T.	T., E.,Mt.	Chronic human sleeping sickness
Pycnomonas	*T. suis*	-	T.	T., E.,Mt.	Disease of young domestic pigs

T.: Trypomastigotes
E.: Epimastigotes
Mt.: Meta-trypanosomes

external genital organs of the male flies (Newstead, 1911). The groups are not only clearly separated by morphological characters but by marked differences in their habitat requirements (Glasgow, 1970). The genus is restricted to the African continent but *G. tachinoides* (Scott, 1939) and recently *G. m. submorsitans* and *G. f. fuscipes* (Elsen *et al.*, 1990) have been recorded on the Arabian Peninsula. The northern limit of their distribution corresponds with the southern edges of the Sahara and the Somali deserts. In the south, no tsetse flies are found south of the northern borders of the Kalahari and the Namibian desert and in the eastern part below 20 - 29° S.

a. the *fusca* group (subgenus *Austenina*) (Haeselbarth *et al.*, 1966)

Most of the species belonging to this group are of little or no economic importance as their habitat is confined to the lowland rain forests (*G. haningtoni, G. nashi, G. tabaniformis, G. vanhoofi, G. severini* and *G. nigrofusca*), the border areas of the forest and isolated relic forests (*G. fusca, G. schwetzi, G. fuscipleuris, G. medicorum* and *G. nigrofusca)* (Fig. 1). All species are difficult to trap, are not attracted by and rarely feed on man (Jordan, 1986). In East Africa, *G. brevipalpis* (the only species of this group used in the present study) is of localised importance and inhabits forest islands often associated with water courses (Ford, 1970; Jordan, 1986) whereas *G. longipennis* inhabits the more arid regions. Morphologically, the members of this group are characterised by superior claspers which are completely free (Newstead *et al.*, 1924).

b. the *palpalis* group (subgenus *Nemorhina*)

The distribution of these species is likewise associated with lowland rain forest but their habitat is extended along river systems in the humid savannah (Fig. 1) (Jordan, 1986). Four of the *palpalis* species were used in this study: *Glossina palpalis palpalis*, *Glossina palpalis gambiensis, Glossina fuscipes fuscipes* and *Glossina tachinoides. G. p. palpalis* and *G. p. gambiensis* occur both in West Africa. *Glossina palpalis gambiensis* occupies an area west of a line from Sierra Leone to North Benin (Challier *et al.*, 1983) whereas *G. p. palpalis* occurs south and east of this line. *Glossina fuscipes fuscipes* occupies the vast area of the West - Central African rain forests and along watercourses in the savannah. In the east, their distribution extends along the shores and river systems of Lake Victoria and Lake Tanganyika. *Glossina tachinoides* (Fig. 2) is distributed along the rivers

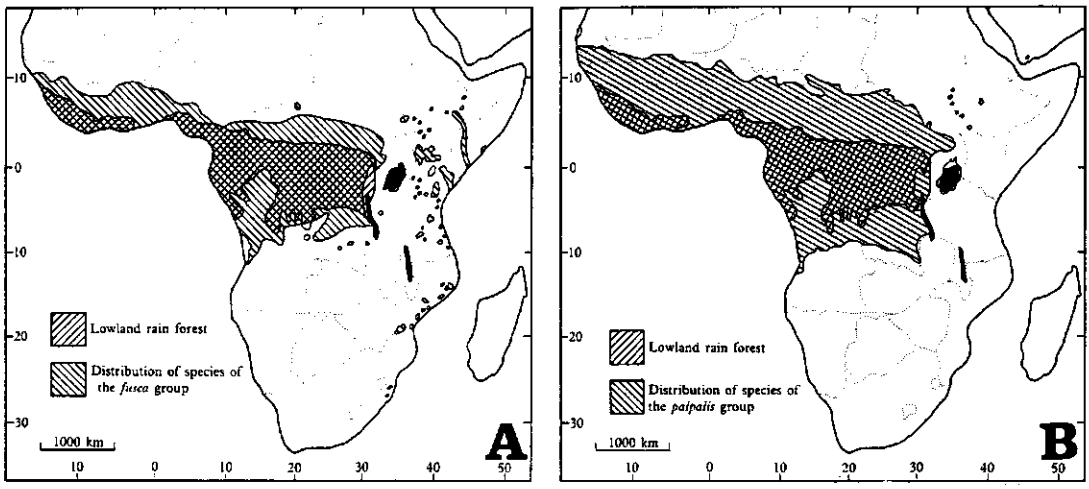

Fig. 1. Distribution of the *Glossina fusca* (A.) and *Glossina palpalis* (B.) group in Africa (after Jordan, 1986).

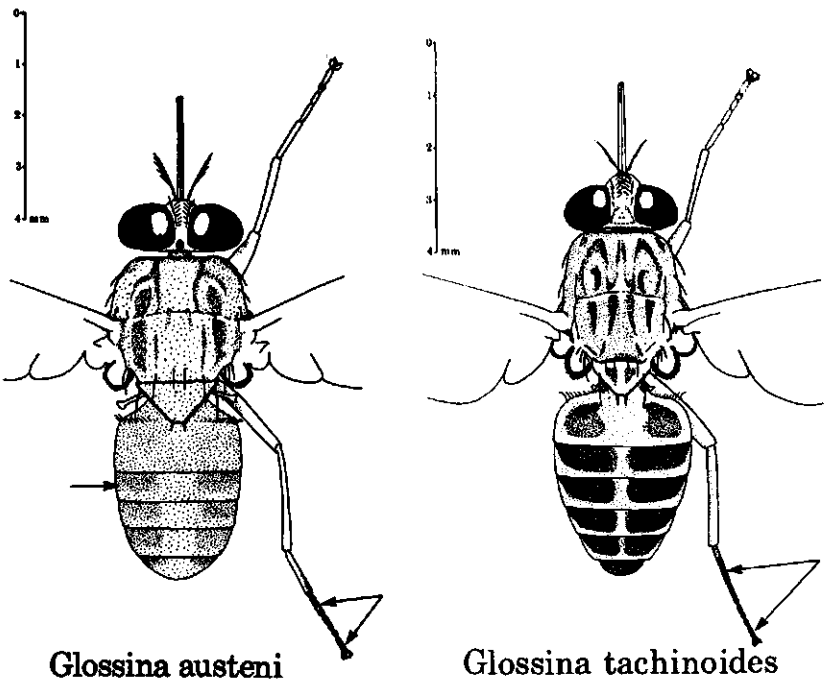

Fig. 2. Dorsal view of female *Glossina austeni* and *Glossina tachinoides* (after: FAO Training manual for tsetse control personnel, Volume 1, 1982)

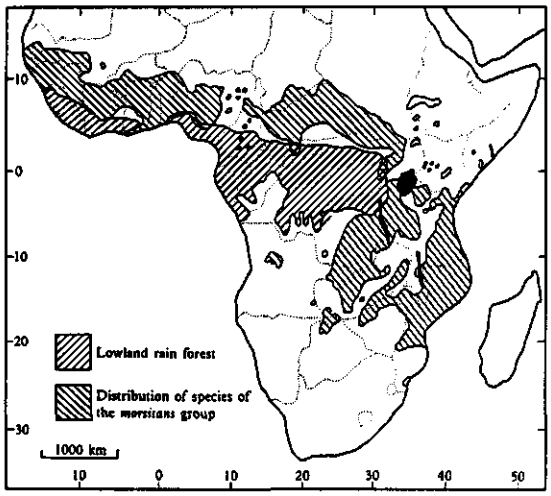

Fig. 3. Distribution of the *Glossina morsitans* group in Africa (after Jordan, 1986).

and streams of West Africa but its distribution extends as far as Ethiopia in the east (Jordan, 1986). The superior claspers of the *palpalis* species are connected by a thin membrane, deeply divided medially. The distal extremities of the claspers remain free and are widely separated (Newstead *et al.*, 1924).

c. the *morsitans* group (subgenus *Glossina sensu stricto*)

All species belonging to this group are restricted to savannah woodlands (Fig. 3) (Jordan, 1986). *G. morsitans* is the most important species in Africa and a major vector of animal trypanosomiasis. Other species are *G. swynnertoni* (limited distribution in Northern Tanzania), *G. longipalpis* and *G. pallidipes*, both occupying thickets and forest edge vegetation. *Glossina austeni* (Fig.1), the only member of this subgenus used in the study, is however considered to be an aberrant member of the group, possessing some primitive characters (Machado, 1959). The fly occurs in secondary shrub, thickets and islands of forest along the coast of East Africa and extends from Zululand in South Africa to Somalia. It is an important vector of animal trypanosomiasis. The distribution pattern of *G. austeni* is scattered and the fly does not occur more than 250 km inland. The superior claspers are completely united by a membrane, fused medially at the distal extremity (Newstead *et al.*, 1924).

3. Tsetse biology

Tsetse flies thrive best at temperatures around 25 °C but interspecific differences have been recorded i.e. *G. m. centralis* can tolerate wider temperature ranges than *G. f. fuscipes* (Buxton, 1955). Adequate moisture conditions are required especially for the larvae, as the water reserve of the freshly born larvae should be sufficient to complete the pupal development. Both sexes of the tsetse flies are obligatory blood feeders. They feed on a variety of vertebrate hosts but the feeding habit of each species is very characteristic and in many cases very selective although no tsetse species feeds on a single host (Weitz, 1970). Many species prefer to feed on Suidae (*G. swynnertoni, G. austeni, G tabaniformes* and *G. fuscipleures*) and Suidae/Bovidae (*G. morsitans morsitans, G. m. submorsitans* and *G. m. centralis*). Other species obtain their food mainly from Bovidae (*G. pallidipes, G. longipalpis* and *G. fusca*) or from Primates and reptiles (*G. fuscipes fuscipes, G palpalis* and *G. tachinoides).* Some animals like zebra, wildebeest and many small antelopes have never been recorded as tsetse hosts (Weitz, 1963).

Glossina spp. are most of the day at rest. Females are only active for a few moments a day and mature males up to 30 minutes a day. Movement of the fly is at random in an uniform habitat with disperse rates of 200 m a week for *G. m. morsitans* (Jordan, 1986) and up to 2000 m a day for *G. p. palpalis* in a linear habitat (Nash & Page, 1953). Tsetse movement and dispersal are in addition related to the climate (temperature, sunshine, wind and rain), the hunger stage of the fly and the sex of the fly i.e. male flies follow hosts without feeding on it ('following swarms') in search for a mate and females are active to find suitable larviposition sites. Most of the flies are diurnal (Buxton, 1955) but some nocturnal activity has been recorded for species like *G brevipalpis* (Harley, 1965) and *G. austeni* (Moggridge, 1948). In some species, males and females have different activity patterns (Buxton, 1955). The inactivity of the tsetse fly and the knowledge on breeding and resting sites has been exploited for control purposes by means of residual insecticides.

Tsetse flies reproduce by adenotrophic viviparity i.e. the egg contains sufficient yolk to sustain the entire embryonic development and the larva in the uterus is nourished by special maternal organs (Hagan, 1951). All nutrients required for the development of the egg up to the adult stage are maternally derived (Tobe & Langley, 1978). The female fly mates on the first or second day after emergence, usually when she takes her first blood meal (Saunders, 1970). In nature, female

flies most likely only mate once, but in the laboratory, multiple copulations have been recorded (Jordan, 1958; Vreysen & Van der Vloedt, 1990). The entire process of spermatogenesis occurs in the pupal stage between day 6 (onset of meiosis) and day 21-23 (formation of mature spermatozoa) (Itard, 1970). Consequently, male flies emerge from the pupae with their entire supply of sperm but become fully potent after 4-6 days. They can successfully inseminate a female fly every 2-3 days, up to 6 times under laboratory conditions (Pollock, 1974; Jordan, 1972). The limiting factor for the mating frequency is the replenishment of the male's accessory glands, required for the formation of a spermatophore (Pollock, 1974). Mating is a prerequisite for a normal ovulation of the first oocyte and the process of ovulation is not stimulated by insemination (Chaudhury & Dhadialla, 1976) but induced by its mechanical stimulus. Saunders & Dodd (1972) suggested in addition, the involvement of chemical endocrine components in the control of ovulation. After mating, viable sperm is stored in the spermathecae for the rest of the female's adult life (Langley, 1977). The oocyte is fertilised when it enters the uterus and the appropriate positioning of the oocyte micropyle versus the opening of the spermathecal ducts are crucial for a successful fertilisation (Roberts, 1972). The female fly displays two ovaries, each containing two polytrophic ovarioles, which are always at different stages of development (Saunders, 1960; Saunders & Phelps, 1970). Eggs develop sequentially (Saunders, 1960) i.e. only one oocyte matures per pregnancy cycle and only one oocyte undergoes vitellogenesis at any given time (Tobe & Langley, 1978). The first oocyte to develop is the internal ovariole of the right ovary (A), followed by a maturation of the internal ovariole of the left ovary (C), which is then followed by maturation of the external right (B) and finally the external left (D). As each ovulation occurs every 9-10 days depending on the temperature, examination of the reproductive status (relative size of the four follicles related to the content of the uterus) allows accurate determination of the physiological age of the female flies (Challier, 1965). The intra-uterine embryonic development is completed within 50-60 hours and results in the hatching of a first instar larva (L1) (Saunders & Phelps, 1970). The intra-uterine development proceeds with the formation of a second instar larva (L2) and is completed with the development of a third instar larva (L3). All larval stages are nourished by lipids produced by the milk uterine gland which is a highly modified female accessory gland. The milk gland is fully ramified at the time of adult emergence and undergoes cyclical changes in the female's adult life which are correlated with the larval development

(Tobe *et al.*, 1973). Feeding on the lipid and proteinaceous secretions of the milk gland is almost exclusively done by the first and second instar larvae with hardly any food being taken by the third instar larva (Bursell & Jackson, 1957). This is corroborated by observations in female *G. austeni* and *G. morsitans,* both showing a peak of gland cell diameter after completion of two third of the pregnancy cycle (Tobe *et al.*, 1973; Denlinger & Ma, 1974; Langley & Pimley, 1975). Development of all three larval instar stages occurs in the female fly except the last part of the third which is spent in the ground. The second instar larva shows the development of the polypneustic lobes which blacken 1-2 days before larviposition. After the larva has been deposited on the ground, the negatively phototactic larva (Parker, 1955) burrows itself rapidly in the soil at a depth of 1-5 cm depending on the species, the season and the soil type (Lewis, 1934). The process of pupation begins with the hardening and darkening of the integument. After the formation of the puparium, the larva retracts itself from the inside of the puparium to form the cryptocephalic pupa (from day two to day four) (Jenkin & Hinton, 1969). On day 5, the head and thoracic appendages are everted and the true (phanerocephalic) pupa is formed. Pupal development takes around 30 days depending on the temperature. After eclosion, the freshly emerged fly will dig its way up in the soil by means of the ptilinum. The thorax of the freshly emerged fly is soft and the abdomen is white when held in the light (teneral fly) (Saunders & Phelps, 1970). Flight muscles begin their development after the first blood meal (Bursell, 1961).

4. Control of the tsetse fly

Many different approaches have been developed and put into practice to keep the disease trypanosomiasis under control such as chemotherapy, chemoprophylaxis, promotion of trypanotolerant breeds of cattle etc.. The most promising and widely used method of trypanosomiasis control however is the control and/or elimination of the vector. Although chemotherapy and prophylaxis are currently still cheaper than vector control (Jordan, 1986), it has no permanent effect on the cycle of the disease i.e. if treatments are relaxed or abolished, the disease incidence will increase again. The trypanotolerance of certain breeds of cattle is limited to exposure of localised trypanosome populations and losses can be severe when exposed to high challenge in a new area or under certain conditions of stress like malnutrition, heavy workload etc. (Jordan, 1986). Vector control remains therefore the most effective method of disease control. A prerequisite for control is a

sound knowledge of the ecology of the tsetse fly. This philosophy was already recognised by C. Swynnerton in 1919 in East Africa and spread and practised in West Africa by T. Nash in the early twenties (Jordan, 1986). A brief account is given of past and present efforts in the control of tsetse in Africa. Control of the parasite is not further discussed here and I refer to Buxton (1955), Hoare (1970 a,b) and Jordan (1974) for reference.

4.1. Non-chemical control methods: clearing of vegetation and game removal

Before synthetic insecticides became available and the more modern approach of traps and targets became commonly accepted in the eighties, the most prevailing form of tsetse control was removal of suitable tsetse habitat or its hosts. In both East and West Africa, ruthless clearing of the vegetation, with the objective of turning woodland or shrubs into grassland, was widely practised (Ford, 1970; Jordan, 1986). Apart from environmental considerations, another disadvantage was the need to slash regenerating bush every year which was either labour intensive, when done by hand, or culminating into high costs in case machinery was used. This destructive method is not widely used any more today, although human expansion and industries have in principle the same outcome, and have evolved into partial clearing where only certain parts of the vegetation (selective clearing) or areas of known fly concentration (discriminative clearing) are removed .

In addition to habitat destruction, the concept of game destruction has been appreciated as a very useful way of controlling tsetse flies and trypanosomiasis, ever since the rinderpest epizootic wiped out enormous amounts of game at the end of the 19 th century. It was widely practised in Botswana, Mozambique, Uganda, Zambia and Zimbabwe before the availability of cheap synthetic insecticides. Although many might view the practice today as distasteful, it was an efficient method of vector control e.g. 20,000 km^2 were made tsetse free in Uganda following a 20-year hunting campaign (1946 - 1966) (Wooff, 1968).

4.2. Chemical control methods

The availability of DDT after the second World War marked the onset of the chemical warfare against the tsetse fly. Several characteristics of the biology of the tsetse fly impose however considerable restrictions

on the use of insecticides for its control (Buxton, 1955): (1) the eggs and a large part of the larval stages are retained in the adult female fly, (2) the pupae are buried in the soil and therefore inaccessible to the impact of insecticides, (3) pupal development may last up to 60 days in colder parts of Africa, (4) the first larva is deposited 14 - 18 days after adult emergence and (5) adults pay only brief visits to their hosts which are mostly wild animals (Jordan, 1986). Therefore, the key to the successful application of insecticides, is to kill all the young emerging flies before they start reproducing. This can be achieved by a single application of a residual formulation or by several adequately spaced applications of a non-residual formulation. In the first case, the insecticide is absorbed through the tarsae of the flies whereas the flies are killed by the contact of the insecticide droplets on their body in the second case. Only three organochlorine compounds (DDT, Dieldrin and Endosulfan) and one synthetic pyrethroid (Deltamethrin) have been commonly used in large scale field operations. Tsetse flies are extremely susceptible to these chemicals which made it possible to use very low dosages with consequent economic advantages and considerable selectivity in favour of other animals (Burnett, 1970). The first chemical to be used was DDT; an extremely stable component with deposits remaining lethal for tsetse up to one year (Baldry, 1963) and easily dispensable in water. DDT was however rapidly replaced by dieldrin, which, despite being more expensive, had a much better persistence of its lethal characteristics in more humid conditions. Both DDT and dieldrin have almost exclusively been applied from the ground for the treatment of the habitat with residual deposits. Both components were replaced by endosulfan for use in spraying operations by virtue of its higher intrinsic toxicity and its better solubility in spray solvents. The most toxic compounds however, so far known for tsetse flies are the synthetic pyrethroids of which deltamethrin has been used for residual spraying (Spielberger *et al.,* 1977), aerosol applications (Molyneux *et al.,* 1978) and for impregnating traps and targets (Laveissière *et al.,* 1981). The major disadvantage are their high costs (1000 times more toxic than DDT but 100 times more expensive) (Jordan, 1986). All above mentioned insecticides can be dispersed as wettable powders, emulsifiable concentrates and as ultra-low-volume solutions. Over the years, the use of insecticides against tsetse has become more selective, which has considerable lessened their pollution of the environment. However, especially the use of persistent insecticides like DDT, Dieldrin and Endosulfan did not prevent the destruction of beneficial non-target insects (Du Toit, 1954) and natural enemies of the tsetse like birds, small mammals etc.

(Matthiessen & Douthwaite, 1985). DDT spraying in Zimbabwe has resulted in accumulation of high levels of DDT residues in wildlife, leading to eggshell thinning of fish eagles and other raptors (Matthiessen, 1985), whereas the use of dieldrin depressed the abundance of insects and insectivorous birds for at least one year (Müller *et al.*, 1981). Even the less polluting method of aerial spraying has negative effects on fish, aquatic crustacea and tree canopy insect species (Matthiessen & Douthwaite, 1985).

4.2.1. Ground spraying of persistent insecticides

Using knapsack pressure sprayers (portable, simple, reliable and robust), persistent insecticides are sprayed selectively at resting sites. The insecticide deposits have to last at least 2 - 3 months and have their maximum effectiveness during the dry season. Consequently, the technique requires a sound knowledge of tsetse distribution, limits of infestations and especially their concentration areas during the dry season. The method fails in humid areas or when the dry season is interrupted with heavy rainfall. Spraying campaigns involve complex logistics and require large amounts of man power. The ability of DDT and dieldrin to accumulate in animal tissue is a negative aspect which requires attention during large scale spraying campaigns. Approximately 200,000 km^2 of tsetse infested land has been reclaimed in northern Nigeria between 1955 and 1978 by the method of selective spraying of resting sites during the dry season. The entire area is considered beyond risk of reinvasion. A total amount of 570 tonnes of DDT, 176 tonnes of dieldrin and 77 tonnes of endosulfan were used during the 22 year campaign (Jordan, 1986).

4.2.2. Aerial spraying of persistent insecticides

Residual insecticides can also be applied from the air for the treatment of vegetation types known to harbour tsetse flies. This technique, which aims for the treatment of night resting sites of tsetse flies in the middle and upper regions of the trees, usually necessitates the use of helicopters in view of the required spray accuracy. As with the ground spraying, insecticide application is likewise restricted to the dry season and operations have to be carried out 1- 3 m above the tree canopy under temperature inversion conditions (early in the morning, late in the evening or at night). Negative aspects are the large quantities of insecticides that have to be used, the huge costs involved in operating helicopters and complex logistics e.g. landing sites have to

be installed at strategic locations limiting the time for refuelling and replenishment of the insecticides etc.. The method does however not require handling of large ground teams of men and equipment.

4.2.3. Aerial application of non-persistent insecticides

An alternative method to ground spraying is the use of fixed wing aircraft (helicopters can also be used but are too expensive) to emit an aerosol of fine droplets containing insecticides over the tsetse habitat. As the droplets are small, they do not leave a persistent deposit in the habitat. The insecticide is dispersed 10-15 m above the tree canopy in swaths of 200-300 m in repeated treatments (5-6 times) with an interval of 9-10 days. Although the method can only be used on flat terrain during temperature inversion conditions and requires sophisticated navigational equipment, it is not restricted to the dry season and does not require the deployment of large ground teams. Absolute perfect weather conditions are a prerequisite for the success with no margin for error (in case of imperfect inversion, mechanical failure etc. the entire spraying cycle has to be restarted). In comparison to spraying of residual insecticides, this technique is less contaminating for the environment and cheaper per km^2. Insecticide drift however remains a problem as the principle is still poorly understood. In the seventies, considerable success was achieved in Botswana, Zambia, Nigeria, Zimbabwe and Uganda using fixed wing aircraft (Davies, 1982; Matthiessen & Douthwaite, 1985).

4.2.4. Chemical control: conclusions

Synthetic insecticides remain until today the major tool for tsetse control as even the widely practised target technology (see below 4.3.1.) remains dependent on the use of insecticides. Recently, considerable concern has been expressed by scientists demanding urgent action for alternatives because of: (1) rapid changes occurring in the target insect population resulting in insecticide resistance, (2) increased costs of developing new insecticides, (3) greater consumers' awareness of residues in food, (4) prohibition in many countries to minimise the use of broad spectrum insecticides (Whitten, 1988). No chemical resistance has been reported so far for tsetse flies by virtue of their slow reproductive capacity resulting in a lower probability of selecting resistant populations. However, the potential exists and has been reported in *G. m. morsitans,* a species capable of metabolising DDT into DDE (Maudlin *et al.,* 1981). In addition, more and more countries are

using insecticides for controlling tsetse flies leading to increased contact of fly populations to sublethal doses of insecticides and increasing therefore the risk of selection for resistance. Alternatives to the use of insecticides are offered by removing the flies from their natural habitat by trapping techniques, biological and genetic control measures.

4.3. Use of attractive devices: traps, targets, animals treated with insecticides

4.3.1. Traps and targets

The first successful use of bait technology against a tsetse species was on the island of Principe (reported by Buxton, 1955). Control efforts against *G. palpalis* were initiated in 1910 by means of squares of black cloth coated with bird lime which were carried by estate workers on their backs. The method was combined with habitat and host destruction in an island-wide approach and resulted in extermination of the fly until it was re-introduced in 1932. The first trap, *sensu stricto,* efficiently used in control efforts against the tsetse flies was the 'Harris' trap (Harris, 1938). The box shaped device (2 x 1 x 1 m), with a V shaped cross section, contained a longitudinal opening along the underside and a fly collecting cage on top. Up to 11,000 traps were deployed in Zululand (South Africa) between 1931 and 1938. During this massive campaign, enormous numbers of *G. pallidipes* were trapped resulting in a reduction of 99.99% of the original *pallidipes* population. Du Toit (1954) however, attributed this enormous success in addition to lack of sufficient hosts following a game destruction campaign and other environmental factors. Another successful early trap was the 'animal trap' or 'Morris' trap designed to suggest the shape of a goat or sheep (Morris & Morris, 1949). Impregnated with insecticides, it was successfully used in a campaign to eradicate *G. p. palpalis* from the island of Principe in 1956-58. These early campaigns clearly demonstrated the feasibility of reducing tsetse fly populations by means of attractive devices, provided sufficient numbers of traps were deployed. The interest in controlling tsetse flies by trapping declined rapidly with the introduction of synthetic insecticides, but was renewed in the seventies, mainly due to increased public awareness of environmental pollution by the excessive use of insecticides. At present, traps and targets are the most widely used devices for tsetse control and have been enormously useful in the study of tsetse behaviour and ecology. The tsetse killing component in targets is still

provided by insecticides, but pollution of the environment is considerably reduced. Trials were carried out in the early seventies with the Langridge Box Screen Trap (Moloo, 1973), the Awning Screen Trap (Swynnerton, 1933) and a modification of the Swynnerton Awning Screen trap (Moloo, 1973). All these traps were more or less efficient in collecting *G. pallidipes*, *G. fuscipes fuscipes* and *G. swynnertoni* (Persoons, 1967; Moloo, 1973). They had in common an opening at the base with a non return device and were designed to attract the fly by its regular dark shape. Once inside the dark interior, the fly would make its way up into the light. Being all very large, they were expensive to construct, cumbersome and time consuming to mount.

A breakthrough came with the development of the biconical trap (Fig. 4a) which was cheap to construct (made out of cloth), collapsible and easily assembled (Challier & Laveissière, 1973). It was designed in such a way that flies were attracted from far away by its shape (a double cone with upper cone white and lower cone blue) and its light colour contrasting with the environment. Lateral openings on the dark surface lead the flies into the upper cone. The trap demonstrated that the shape of an animal was no prerequisite for attracting tsetse flies. The Challier-Laveissière trap proved to be highly effective for trapping *palpalis* species and captured about 10 times more flies than the 'Morris' trap, the previous standard trap for riverine species (Green, 1994). Many efforts have been undertaken to improve the original version of the biconical trap. The current used version has a 'royal blue' lower cone, white mosquito netting for upper cone and interior black targets (Challier *et al.*, 1977). In addition, a number of variants have been designed and tested (Fig. 4 b-d): the monoconical trap, a cheaper version of the biconical, designed for the control of *Glossina fuscipes quanzensis* (Lancien, 1981), the pyramidal trap, another cheaper alternative designed for the control of *G. p. palpalis* and *G. f. quanzensis* in the Congo (Gouteux & Lancien, 1986), the Vavoua trap, designed for the control of *G. p. palpalis* in Ivory Coast (Laveissière & Grébaut, 1990), the bipyramidal trap, developed for use against *G. fuscipes fuscipes* (Gouteux, 1991) and the monoscreen trap for the control of *G fuscipes* in mid- and central Africa (Dagnogo & Gouteux, 1985; Gouteux & Sinda, 1990).

The savannah tsetse species of East Africa, responded however much less to the biconical trap and its derivatives. Simultaneously to the work in West Africa, very efficient traps (Fig. 5) have been developed and tested in Zimbabwe for trapping *G. pallidipes* and *G. morsitans morsitans* i.e. the vertical vane trap, a trap with a dark body surrounded by vertical V shaped vanes made of gauze (Hargrove, 1977), the beta

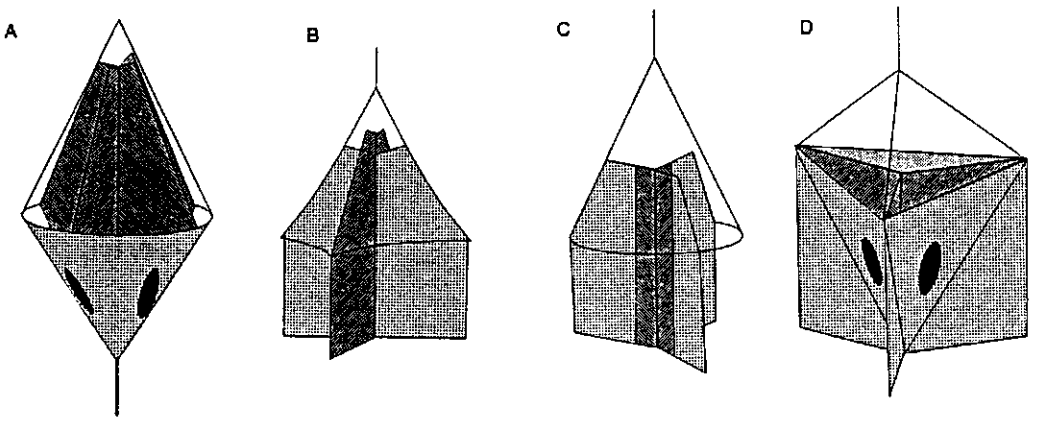

Fig. 4 Diagrams of traps designed for *palpalis* species. (a) Biconical (Challier & Laveissière, 1973), (b) pyramidal (Gouteux & Lancien, 1986), (c) Vavoua (Laveissière & Grébaut, 1990), (d) bipyramidal (Gouteux, 1991).

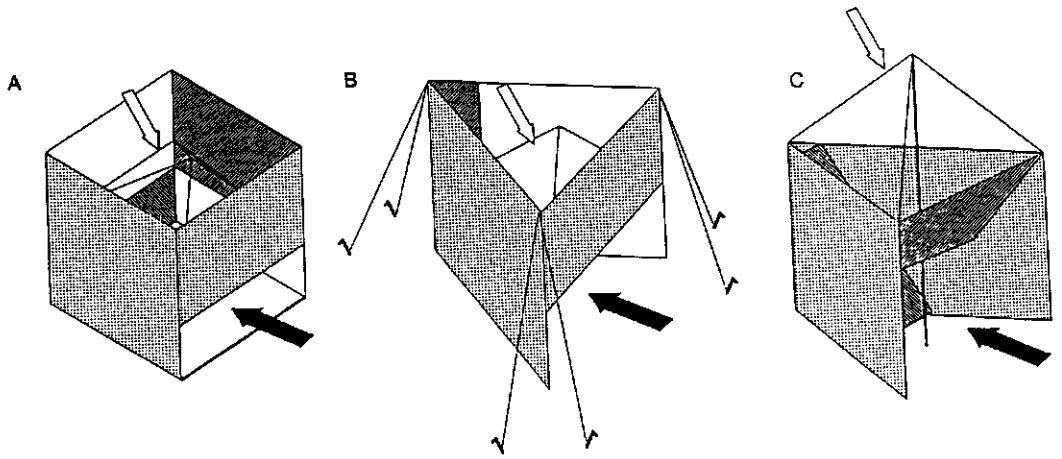

Fig. 5 Diagrams of traps designed for *morsitans* species. (a) F3 (Flint, 1985), (b) Epsilon (Hargrove & Langley, 1990), (c) NG2B (Ngu trap) (Brightwell *et al.*, 1987).

trap (Vale, 1982), the cubic F2 and F3 trap, which catches 9 times more *G. pallidipes* than the biconical (Flint, 1985) and the Epsilon trap, a cheaper version of the F3 (Hargrove & Langley, 1990). The Ngu trap, based upon the F3 trap, was developed for *G. pallidipes* in Kenya. Baited with acetone and cow urine, it was 3 times more effective as similar baited biconical traps (Brightwell *et al.*, 1987). The F3 and Ngu are likewise the preferable sampling tools for *G. longipennis* (Kyorku *et al.*, 1990).

Based upon the attractivity of the lower blue cone of the biconical trap, and the realisation that only a very brief contact with the insecticide impregnated surface of a trap is required to kill the fly, squares of blue cloth impregnated with insecticides have been designed by tsetse workers as a cheap but efficient alternative for complicated tsetse traps. Various designs have been tested (Fig. 6) and used in control efforts both in West and East Africa such as the simple blue target (Laveissière *et al.*, 1980), the Vavoua target (Laveissière *et al.*, 1987), blue/black/netting target (Mérot et Filledier, 1985), S-type target consisting of a central black square of cloth flanked by 2 panels of black mosquito netting (Vale *et al.*, 1985) and the 'all cloth' S-type target (Willemse, 1991). In the past decade, insecticide impregnated targets and traps have become the most commonly used tool in the control of tsetse species. Some of the earliest successes against tsetse flies using bait technology were achieved in the savannah regions of West Africa where the distribution of the *palpalis* species is virtually linear along riverine forest eco-systems. Dispersion of the flies can be rapid but remains mostly limited to the riverine habitat. Simple blue cloth screens, placed at the river bank or suspended from trees fringing the river and deployed at a density of 7 to 11 per linear km achieved between 88% and 99.1% control of *G. p palpalis, G. p. gambiensis* and *Glossina tachinoides* in Nigeria (Oladunmade *et al.*, 1985), Burkina Faso and Ivory Coast (Mérot *et al.*, 1984; Küpper *et al.*, 1984). Likewise, similar reductions in fly population were obtained with biconical traps in Nigeria and Burkina Faso (Takken *et al.*, 1986; Laveissière *et al.*, 1980). Despite very encouraging results, in none of the above cases was eradication achieved. Only Küpper *et al.* (1982, 1984) reported 100% control against riverine species in Ivory Coast by the use of biconical traps.

Control efforts against *palpalis* species in the more humid forest regions of Africa have been more difficult as the distribution of the flies becomes more widespread. High densities of targets and traps are required in localised areas to obtain the desired effect. Simple blue cloth targets, deployed at 250 per km^2 in Ivory Coast reduced the *G. p.*

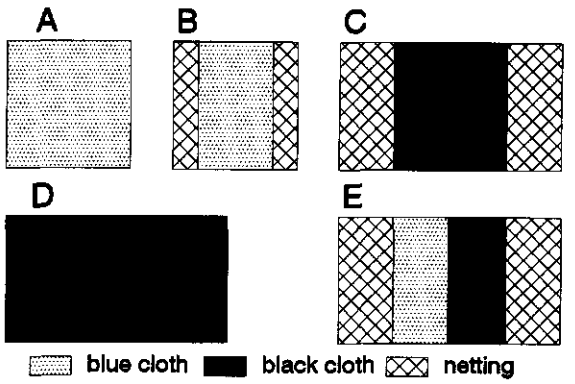

Fig. 6 Diagrams of insecticide impregnated targets. (a) blue target (Laveissière *et al.*, 1980a), (b) Vavoua target (Laveissière *et al.*, 1987), (c) S type target (Vale *et al.*, 1985), (d) all cloth S target (Willemse, 1991), (e) Blue/black/netting (Mérot & Filledier, 1985)

palpalis population by 90% (Laveissière *et al.*, 1980). In a later trial, similar targets placed at 186 per km^2 over 86 km^2 reduced the population between 90 and 99% (Laveissière *et al.*, 1986). In the Congo, 90% control was achieved against *G. p. palpalis* and *G. f. quanzensis* by placement of 330 pyramidal traps in 55 villages (Gouteux & Sinda, 1990). During an emergency programme in Uganda against *G. fuscipes fuscipes*, 12,000 pyramidal traps achieved 99% control after 5-7 months in the Busoga and Tororo districts (Lancien, 1991). Control campaigns of these magnitude require a high level of organisation, investment, a good geographical survey and a perpetual effort (with the participation of the local communities) to re-impregnate the targets and clearing of the vegetation in the near vicinity of the bait devices (Green, 1994).

The large and extremely mobile tsetse populations of the savannah (*morsitans* group) require highly efficient baits covering large areas (Green, 1994). An enormous amount of research has been conducted in East Africa with respect to attraction of savannah species to host odours. Components of the breath of host animals (carbon dioxide, acetone, octenol, ketones) (Vale, 1974), urine (phenols) (Owaga, 1984; Dransfield *et al.*, 1986) and skin secretions (sebum) (Warnes, 1990) increased trap catches significantly. The availability of these attractant odours against these species has been instrumental in the success of many control operations. Various models of targets deployed at 4-8 targets per km^2 obtained between 97% and 100% control against

G. pallidipes, G. morsitans morsitans and *G. morsitans centralis* in Zambia and Zimbabwe (Vale *et al.,* 1986, 1988; Willemse, 1991). In the Nguruman area in Kenya, 100 Ngu (NG2B) traps baited with acetone and cow urine, reduced the *G. pallidipes* and *G. longipennis* population with 98% and 90% respectively over a 9 month period in 100 km^2 (Dransfield *et al.,* 1990). Odour baited blue and black insecticide targets, deployed at a density of 3-10 km^2, removed *G. morsitans submorsitans* from 600 km^2 in Ethiopia (Slingenbergh, 1992). These examples amply demonstrate that good control and/or eradication of *morsitans* species can be obtained with odour baited traps and targets deployed at densities of 4-10 km^2 provided the control campaigns are well organised and careful precautions are taken against re-invasion (Green, 1994).

4.3.2. Pour - on applications

The development of long lasting formulations of synthetic pyrethroids has opened potential for use on natural baits i.e. the livestock itself. Successes have been obtained as formulations in cattle dips in Zimbabwe (Thomson *et al.,* 1991) and as a 'pour on' formulation against *palpalis* species in Burkina Faso (Bauer *et al.,* 1992) and *G. austeni* on Zanzibar (Höreth-Böntgen, 1992).

4.4. Biological control methods

4.4.1. Pathogens

The list of micro-organisms pathogenic to tsetse is limited and the knowledge is rudimentary. The potential of using pathogens in tsetse control is low as the larval stage, for many insects a highly susceptible stage for pathogens, is cryptic and the pupae are protected by a hard pupal shell. Intracellular rickettsia-like micro-organisms, observed in the midgut eptithelium, fat body and developing oocytes of various tsetse species, are potential candidates for biocontrol. They were associated with host cell disruption and degeneration (Pinnock & Hess, 1974). Salivary glands of 1% of male *G. pallidipes* collected in the Kibwesi forest in Kenya were abnormally enlarged. Virus like particles were found in the epithelial cells of these hyperplastic salivary glands and the condition is often associated with complete sterility (Jaenson, 1978). The virus is transmitted transovarially and can be present in a latent condition in some apparently normal flies (Jaenson, 1986). The

characteristics of the virus were studied but can so far not be placed in any of the existing taxonomic groupings of DNA viruses (Jordan, 1995). Six genera of pathogenic bacteria have been isolated from field collected *G. pallidipes* flies. Feeding of these bacteria to laboratory reared *G. m. morsitans* caused high mortalities (Kaaya & Okech, 1990). Other pathogenic organisms of adult tsetse flies are various genera of bacteria (Nash, 1970; Jordan, 1995), various Nematodes (Laird, 1977) and several strains of species of entomopathogenic fungi (Jordan, 1995). Although these pathogens can cause high mortality in the laboratory, the practical use for control purposes i.e. the development of efficient methods of introducing these pathogens into the wild fly population, remains problematic and requires further investigation.

4.4.2. Parasites

Only a few unsuccessful attempts to release biological control agents into tsetse habitats are known. One of them was the release of ± 300,000 *Nesolynx* (Hymenoptera parasite of tsetse pupae) in Malawi (Lamborn, 1925) and close to 14 million in Tanzania (Nash, 1933). Both trials achieved little. Ten species of the genus *Exhyalanthrax* (*Thyridantrax)* (Diptera: Bombyliidae) are known parasites of pupae with high rates of parasitism in East and South Africa. However, no suitable method for mass production has been developed and consequently, the flies have never been used as biological control agents. As Jordan (1986) concluded: " Some of these natural enemies can cause high mortality in tsetse flies, but the difficulty lies in boosting this into the advantage of man."

4.4.3. Predators

Numerous animals have been reported to be predators of tsetse flies or their pupae, but the records are mostly anecdotal: mammals (baboons, bats, mongoose, shrews etc.), birds (guinea fowl, francolins), insects (dragonflies, beetles, crickets, ants etc.) and spiders. Rogers & Randolph (1990) reported a pupal mortality rate of 23% over a 30 day development period for *G. pallidipes.,* a mortality rate which was independent of pupal density. Very little is known about the biology and ecology (habitat, longevity, breeding periods, frequency of their meals etc.) of these predators and their relationship with the tsetse fly, nor is there any evidence of a specific action of these predators towards tsetse (Laird, 1977).

4.5. Genetic control methods

Genetic control methods are all based on introducing sterility in an insect population. There are 4 existing methods to introduce sterility i.e. hybridisation of closely related species or subspecies, chemo-sterilisation, radio-sterilisation and genetic manipulation (Itard, 1974). Only the approach of introducing deleterious genes and chromosomes into a native tsetse population has never received a practical follow-up (Curtis, 1969).

4.5.1. Hybridisation

The first genetic control attempt against a tsetse species was based upon the hybridisation capacity of closely related species. The possibility of controlling tsetse populations by hybrid sterile males was already proposed in the early forties (Vanderplank, 1944), long before the concept of SIT was tested. Although numerous publications have been devoted to the mating behaviour of closely related *morsitans* and *palpalis* species and unravel the mechanism of hybrid sterility resulting from those crosses (see also part 2 of this thesis), only 2 small field tests have been conducted. In 1945, Jackson (1945) introduced two exotic species (*G. morsitans morsitans* and *G. fuscipes fuscipes)* in the habitat of *G. swynnertoni. G. morsitans morsitans* survived to produce a second generation (the habitat of both species overlap and they produce sterile cross matings (Ford, 1971) but *G. fuscipes fuscipes* failed to do so, probably because they could not find suitable larviposition sites . Likewise, Vanderplank (1947) comments on a similar experiment which showed the possibility of eradicating *G. swynnertoni* by releasing large numbers of *G. m. centralis.* Despite the fact that the principle has not been tested further in the field, it remains an intriguing concept and the knowledge gained in the laboratory deserves further experimentation and validation in the field.

4.5.2. The sterile insect technique (SIT)

The sterile insect technique has been used successfully against various species of tsetse flies and relies on the rearing in large numbers of the target insect, the sterilisation of one of the sexes by means of ionising radiation or chemosterilants and the sequential release of the sterilised insects in the habitat (Williamson *et al.,* 1983 a,b,c,d; Politzar & Cuisance, 1984; Oladunmade *et al.,* 1990). The research

presented in this thesis deals with ionising radiation and its effects on tsetse biology and therefore, a detailed overview is given of the use of SIT in control of tsetse flies and other pest insects.

5. SIT and pest insects

5.1. An historic overview

The New World Screwworm

The SIT approach has been developed and applied successfully for several insects. The most successful and best known programme is the eradication of the New World Screwworm (NWS) (*Cochliomyia hominivorax)* from Curaçao, south-east USA, Puerto Rico, Mexico (Snow, 1988) and Libya (Lindquist & Abusowa, 1991, 1992). The screwworm larva is a major parasite of livestock and other warm blooded animals including humans. It was an ideal target insect as rearing methods could be developed, natural populations densities were low, the female fly only mates once (sperm competitiveness was not an issue) and a low dose of gamma rays (70 Gy) was required to sterilise the flies with little somatic damage. The initial reduction of the fly population was achieved by treatment of wounds and reduction of oviposition sites by limiting branding, dehorning, castrating etc. (Snow, 1988). Between 1935 and 1937, extensive control measures were undertaken in the US i.e. intensive animal inspection, combined with wound treatment and good animal husbandry greatly reduced the incidence of wound infections, but eradication could not be achieved because wildlife and non-attended livestock served as reservoirs (Novy, 1991). The first successful SIT trial was directed against a completely isolated screwworm population on the island of Curaçao (Baumhover *et al.,* 1955). Sterile flies were released at densities of 300 flies km^2 and eradication was achieved within 6 months after the initiation of the trial. This prompted a large scale attempt in Florida (5,000 km^2) which culminated in the eradication of the screwworm over the entire south-east and south-west of the United states. From 1962 to 1980, between 40 to 200 million sterile flies were released per week, all produced in the production facility in Texas (Novy, 1991). Although various factors contributed to the success of the programme in the US such as permanent availability of sufficient numbers of sterile flies, an active information and education programme accompanied by distribution to livestock owners of maggot collection kits, a key factor for its success was the financial support and full participation of the

ranchers during the entire programme (Snow, 1988). It has been estimated that the economic benefits for livestock production in the USA from screwworm control between 1962 and 1976 exceeded US$ 1,000 million (Novy, 1991). The programme has subsequently been expanded into Mexico and further south in Central America. To date (1995), Mexico is free of screwworms.

In 1988, the NWS was discovered by veterinarians in the north-west Libyan Arab Jamahiriya. It was most likely introduced from the Americas although the mode of introduction remains unknown. The presence of the NWS in Libya was viewed as a potential major disaster for North Africa, the Mediterranean Basin and the whole of sub Saharan Africa (Lindquist & Abusowa, 1991). The cost of controlling and inspecting the disease was estimated at US$ 250,000 per year. The total infested area was estimated at 25,000 km^2 by the end of 1990. It was decided that the NWS had to be eradicated by the SIT, the only eradication technique available. After confirming the mating compatibility between the Libyan strain and the Mexican NWS strain, an eradication strategy was developed. The sterile males were produced in the NWS mass rearing plant in Tuxtla Gutierrez, Chiapas, Mexico and transported by air to Libya. Releases were initiated in December 1990 with the dispersal of 3.5 million flies/week over the total infested area. The full eradication phase was initiated in February '91 with the release of 28 million flies/week. By May 1991, this number was increased to 40 million/week treating a total area of 40,000 km^2 (including a 15,000 km^2 biological barrier). A total of 1,300 million sterile flies were released between December 1990 and October 1991. (Lindquist & Abusowa, 1992). The fly was declared eradicated in 1992.

Fruit flies

After the successful use of the SIT technology against the screwworm fly, the method was transferred to other pest insects, mainly fruit flies and various species of Lepidoptera. Fruit flies are serious economic pests. They are relatively easy to rear in large numbers and therefore good candidates for SIT programmes. The first test against the Mediterranean fruit fly (MFF) (*Ceratitis capitata*) was conducted on Hawaii in 1959 - 1960 where almost 19 million sterile fruit flies were released during a 13 month period. The sterile flies, irradiated with 100 Gy in the late pupal stage, reduced the native population with 90%. Although eradication was prevented by invasion from other infested areas, the test proved the feasibility of suppressing wild fruit flies with sterile males (LaChance *et al.*, 1967). In current programmes, MFF

pupae are irradiated under anoxia, 48 hours before adult emergence with a dose of 140 Gy. Prior to the release, the pupae are placed in paper bags at densities of 20,000 per bag together with some sugar as a food source for the emerging flies. The bags with the flies are released by aircraft when 80% of the pupae have emerged (Lindquist *et al.*, 1990). A major SIT programme was initiated in Mexico following the immigration of the MFF from Guatemala in 1977. The final objective is the eradication of the fly from Mexico and the whole of Central America. The fly was eradicated from 8,000 km^2 by 1982 by a combination of bait spraying with malathion and the release of 500 million flies per week. The estimated benefits of the programme are close to US$ 1,000 million per year (Lindquist *et al.*, 1990).

Lepidoptera

One of the major pests of apples and pears is the codling moth (*Cydia pomonella*) which has been controlled in the past by chemical means. This however, leads invariably to outbreaks of other insect pests such as spider mite and leaf rollers, probably due to the toxic effects on natural enemies of harmful arthropods. Twenty years of research on the codling moth have resulted in sophisticated rearing and release methods. The moths are irradiated with 350 Gy inducing 92% and 100% sterility in male and female moths respectively (Dyck *et al.*, 1993). Feeding is done on a sawdust diet, which includes a Calco red dye. Emerging adults have their internal organs coloured red, which can be used to discriminate against wild moths (yellow intestines). Chilled moths are released by specially designed dispensers mounted on dune buggies or motorbikes driven through the orchards. The desired ratio of sterile to wild moths is 40 to 1. Monitoring is done by pheromone traps set out once a week for one night and by close inspection of the fruit on the trees. During a pilot trial (1976 - 1978), the codling moth was eradicated from 500 ha of apple orchards in the Similkameen valley, British Colombia, Canada. Currently a large scale programme (8,000 ha of orchards) is being conducted, including 2 years of prior suppression by sanitation and insecticides, followed by 3 years of releases at a rate of 3 - 5 million moths per week (Dyck & Gardiner, 1992).

Another serious Lepidoptera pest is the pink bollworm; a tropical insect that has invaded all cotton producing areas in the US since 1928 (Snow, 1988). Like the codling moth, it is relative easy to control, but application of insecticides leads to infestations of *Heliothes* (Snow, 1988). Initially, the eradication programme did not include a radiation component owing to the high doses required to sterilise females after

mating and the sensitivity of the midgut to radiation. Therefore, diapause weevil control with insecticides was combined with trapping of the adults using pheromones. Currently, high quality sterile weevils are obtained by irradiation of males and non-mated females with the non-debilitating dose of 100 Gy whereas gravid females are sterilised by dipping in Dimilan® (Snow, 1988). Currently, 35 million pink bollworms are reared per week in Phoenix, Arizona and released by air in the San Joaquin Valley in California obtaining sterile to wild ratios of 200:1 (Lindquist *et al.,* 1990).

A major problem with Lepidoptera is the high radiation dose required to obtain complete sterility. Irradiating the parentals however, with sub-sterilising doses, renders their F1 offspring completely sterile with a sex ratio in favour of males. This F1 or inherited sterility has been used against the gypsy moth (*Lymantria dispar),* a pest of hardwood trees in North America. Pupae are sterilised with 150 Gy or male pupae, treated with 100 Gy are mated with normal females and their offspring eggs kept in diapause until the next spring. This method, which synchronises the hatch of the sterile moths with the one of the native population, eradicates every year isolated outbreaks of the gypsy moth (Lindquist *et al.,* 1990).

Other successful SIT programmes have been conducted against:

1. the Melon fly (*Dacus cucurbitae*) whose larvae are serious pests of fruits and vegetables. Pupae are irradiated 3 days prior to emergence with 70 Gy and released as adult flies in bags or in free release methods. A SIT programme was initiated in Japan in 1972 to eradicate the fly from Kume and adjacent islands by a combination of population suppression with lure/toxicants and the release of sterile flies (6 - 20 million per month). In 1984, the programme was extended to the Mikao island group. Eradication was achieved in 1976 and 1987 for the two island groups respectively.
2. Mexican Fruit Fly (*Anastrepha ludens).* This serious pest of citrus trees in the southern US is kept under control by the release of 20-30 million flies per week using free release techniques from aircraft. These releases are supplemented with the dispersal of 2 million flies per week from the ground in Mexico (Lindquist *et al.*, 1990).
3. the Onion fly (*Delia antiqua*), a major pest of onion, is controlled in the Netherlands solely by the release of sterile flies. The flies can be purchased from a private company and up to 170 million flies are released each year (Lindquist *et al.,* 1990).

Tsetse flies

Chemosterilisation

Sterilisation of a tsetse species (*G. morsitans*) with chemosterilants (apholate and metepa) was first attempted in the early sixties but without much success: the treated flies were only rendered sub-sterile and suffered from excessive mortality rates (Chadwick, 1964). Fine-tuning of the methodology resulted in more acceptable results. *G morsitans* flies were completely sterilised by (1) walking of adult flies on glass surfaces treated with 10 mg of tepa/ft^2, (2) spraying of adult flies in a wind tunnel with 0.25 ml of a 5% solution of tepa or (3) treating pupae by a 60 sec. emmersion in a 5% solution of tepa (Dame & Schmidt, 1970). The use of chemosterilants offers opportunities to treat portions of the native insect population which would have the advantage of avoiding the problems of rearing and releasing large numbers of sterilised males. On the other hand, it would require very efficient trapping devices for the auto sterilisation (Knipling, 1969). Moreover, active chemosterilants degrade rapidly in the field and the flies might develop resistance to the sterilants (Dame & Schmidt, 1970). The method was pioneered in the field on an island in Lake Kariba, Zimbabwe in the late sixties with field collected *G. m. morsitans* pupae, sterilised by 30 - 60 minutes exposure to deposits of 10 mg of tepa/ft^2. Prior to the releases, two lindane applications, separated by 30 days, reduced the natural population (estimated at 4,000 male flies/mile^2) with 50%. However, after 6 months of releases, the native fly population was not eradicated mainly because survival of the sterile males was only 17% of that of the native males and a maximum ratio of only 0.12 sterile to 1 wild male was obtained. The poor survival of the male flies in the field was attributed to an inhibition of the fly musculature (Dame *et al.,* 1968). Therefore, a second trial was conducted on the same island but now the option of 'pupal releases' was taken. Fly population (3000/mile^2) was reduced with 2 aerial applications of dieldrin, followed by release of pupae, sterilised by dipping in a 5% solution of tepa. 98% control was achieved after 9 months. It was concluded that the competitiveness of flies sterilised and released as pupae was much higher than that of released adults (Dame & Schmidt, 1970). Problems during the programme were inadequate supply of field collected pupae for sterilisation and stability of the chemosterilant. Furthermore, the chemosterilants used proved to be highly toxic, in particular for mammals. Recently, the concept of sterilising native fly populations has received new attention

due to the availability of new chemicals, acting as disruptive agents in the process of embryogenesis and metamorphosis. Topical application of 2 ng of pyriproxyfen, a juvenile hormone mimic, sterilised female *G. m. morsitans* flies for life (Langley *et al.*, 1992). Non viable pupae were likewise produced by female *G. m. morsitans* exposed by tarsal contact with netting treated with an oil formulation of pyriproxyfen (Langley *et al.*, 1992). These components have the advantage that they act complementary to the use of pyrethroids in traps and targets. A small trial was carried out in Zimbabwe, where 41 odour baited Epsilon traps were treated with pyriproxyfen. Tsetse entering the trap were not retained but were treated with the hormone mimic during their escape effort. Emergence rates of pupae collected in the area dropped to 30 and 2.7% for *G. pallidipes* and *G. m. morsitans* respectively (Hargrove & Langley, 1990). Although these first results were very encouraging, its value for tsetse control will depend on the attractiveness of the device used, the residual activity of the chemicals in the field and the issue of transfer of the component to the female flies during mating.

Radiosterilisation

Radiosterilisation of a tsetse species was first carried out by Potts (1958) who obtained a high degree of sterility by exposing *G. morsitans* pupae to 60 - 120 Gy of gamma radiation. Field studies analysing the effects of transport, irradiation etc. on the quality of gamma sterilised male flies belonging to the *palpalis* group, were initiated in West Africa in 1972 (Chad) (Cuisance & Itard, 1973). Male *G. tachinoides* flies, reared and gamma sterilised with 150 Gy in Maison Alfort (France), were placed in Roubaud cages at a density of 100-150 flies per cage and shipped in isothermic boxes (25 °C) by air mail to Chad (transport time between 7 and 17 hours). Upon arrival at the airport, they were immediately fed on rabbit ears, left for several hours to rest and then transported by car or boat to the various release sites (Cuisance & Itard, 1973). The study revealed that the irradiation treatment reduced the percentage of take-off during the release sessions but had no detrimental effect on fly survival. It was likewise shown that the activity pattern of the sterile flies was slightly disturbed during the first 48 hours after release. This was mainly caused by the rearing and handling methods whereas the irradiation treatment had no visible effect. However, from day 3 on, the laboratory reared males displayed the same behaviour as the wild males. The ratios of sterile to wild males did not exceed 0.4 and consequently, the impact on the natural population was negligible (Cuisance & Itard,

1973). This small experiment showed the feasibility of transporting adult male flies of a rather fragile species over long distances without any significant negative impact on their quality and behaviour.

Other pilot studies, conducted in Burkina Faso, demonstrated the benefits of prior suppression by application of a non-residual insecticide on the outcome of a SIT programme (Cuisance *et al.*, 1980a). Sterile male *G. p. gambiensis* were released in optimal ratios (7 to 10) in a native population with and without prior application of a non residual insecticide. In the area with prior suppression, the fly was eradicated after 19 months, whereas an additional 5 months were needed to achieve the same in the area without prior insecticide application (Cuisance *et al.*, 1980a). The prior application of insecticides did reduce considerably the numbers of sterile males and the time required to achieve eradication and this implied reduction in costs. Further economic savings can be obtained by optimising releases in terms of time spacing (7 days versus 10 days) and release sites (2 km versus 200 m apart) (Politzar & Cuisance, 1982).

Another small release trial was carried out in the Volta Noire source tributaries (S-W part of Burkina Faso), under the auspices of WHO to control a massive outbreak of human sleeping sickness (Van der Vloedt *et al.*, 1980). During the dry season of 1977/78, two spraying operations of a synthetic pyrethroid (decamethrin), separated by 14 days, were applied from a helicopter and followed by the release of ± 5,500 sterile male *G. p . gambiensis* in 10 release sessions. A 95% reduction of the original native *G. p. gambiensis* population was obtained following the insecticide application. Wing fray analysis and ovarian ageing revealed that older males (WF > 2) and females older than age group III had disappeared. A small proportion of the younger parous females however, survived at least one of the treatments. Some interesting aspects of the performance of the released male flies were revealed during the relatively short monitoring period of 33 days: (1) dispersal rate up to 2000 m after 48 hours, (2) good survival with maximum periods between release and recapture of 20-44 days, (3) good response to the monitoring device (30 % recapture rate with Challier biconical trap). During the entire trial period, on average 8.7 sterile males were trapped for each wild male (Van der Vloedt *et al.*, 1980).

Results of these feasibility studies culminated in larger scaled integrated eradication campaigns in East and West Africa. The first SIT programme of any magnitude was conducted against *G. m. morsitans* in the Tanga area, Tanzania. Major contributions were made in the development of procedures for sterilisation, handling, packaging and

releasing of the sterile males. In addition, a facility was created to maintain a colony of 60,000 females, the first of its kind in Africa. The major objective of the programme was to evaluate the SIT methodology against a tsetse species and to test the concept in the field (Williamson *et al.,* 1983a). During the 15 month trial period of active releases, a stable colony of 50,000 *G. m. morsitans* females was successfully maintained. The option of releasing pupae rather than adult flies was taken, as the experimental release area (Mkwaja Ranch) was free of human sleeping sickness. Tsetse flies are most susceptible for trypanosome infections following the first blood meal; pupal releases therefore might increase the potential risk that sterile flies contribute to transmission. This reduced in addition the complexity of the handling procedures. During the programme, efficient and sophisticated pupal handling and irradiation techniques were developed and successfully implemented. 48% of each puparial batch was retained after the female emergence flush and chilled at 10 °C for 1 to 4 days. After irradiation (dose of 118 Gy in a nitrogen atmosphere), the pupae were packed in pre-cooled freezers and transported to the release sites in the field. Quality of the pupae stored at 10-11 °C was not impeded as long as a relative humidity of 70-90% was maintained. At the test site, the pupae were placed in field emergence cages and buried under sand mixed with a fluorescent day glo powder, which automatically marked the flies during emergence. The puparia warmed up very rapidly, teneral flies started emerging after 10 min. and eclosion was completed within 1 hour. Losses due to storage (2.9%), radiation (1.4%) and transport (4.2%) were minimal (Williamson *et al.,* 1983b). To prevent re-invasion, a 1 km wide fly barrier was cut surrounding the entire experimental area (195 km^2). Clearing was done both by hand, bulldozers and brushcutters at an average cost of US$ 37 per ha. The fly density of *G. m. morsitans* was estimated at 630/km^2 in the test site (Gates *et al.,* 1983). Prior to the release of sterile males, the *G. m. morsitans* native fly population was completely wiped out by two aerial applications of a non residual insecticide (endosulfan) used as a 20% ULV aerosol formulation with an interval of 28 days (Williamson *et al.,* 1983c). Sterile males were released as pupae at a rate of 135/km^2 resulting in an average ratio of 1.12 sterile to 1 wild male. This low ratio was however enough to keep the indigenous population at the 80 - 95% reduction level obtained after the insecticide applications. Migration of wild flies from outside the experimental area prevented the eradication of the test species (Williamson *et al.,* 1983c).

A combination of trapping techniques, insecticide impregnated screens (IIS) and the release of sterile males was used to eradicate *G*

p. gambiensis and *G. tachinoides* from a total area of 3,500 km^2 in the Guinea savannah, south of Bobo Dioulasso in Burkina Faso. Prior suppression of the native fly population was achieved by placing 6,500 IIS along 650 km of linear gallery forest for 4 months during the dry season (Politzar & Cuisance, 1984); an approach which avoided the spraying of a non-persistent insecticide for population reduction (Van der Vloedt *et al.*, 1980). Screens were treated with 200 mg of deltamethrin and placed at intervals of 100 m. These operations were followed by releases of sterile males in the rainy season. To supply the required number of sterile male flies, a colony of 150,000 *G. p. gambiensis* females and 85,000 *G. tachinoides* females was maintained in mass rearing facilities using the *in vitro* feeding technique. The use of the IIS reduced the native *G. p. gambiensis* and *G. tachinoides* populations with 91 and 94% respectively. This enormous reduction in the native fly population reduced the requirements of sterile males to only 20-35 sterile males per linear km to obtain the desired ratios of 10:1. The main river systems were free of tsetse 6 months after the release of sterile male flies (Politzar & Cuisance, 1984).

These successes in Burkina Faso were repeated in Nigeria where the use of IIS, traps and the release of sterile males eradicated *G. p. palpalis* from a total area of 1,500 km^2 in Southern Plateau State. The flies were reared at a production centre in Vom, situated on the tsetse free Jos plateau. Both *in vivo* and *in vitro* feeding techniques were used to maintain colonies through the entire project period. Feeding of the *in vivo* colony was done on guinea pigs (Oladunmade *et al.*, 1990) and this colony reached its maximum size of 60,000 flies in 1986. On average, 4,700 sterile males were released per week from the *in vivo* colony. These were supplemented on a weekly basis with approximately 3,500 sterile males from the *in vitro* colony. The maximum *in vitro* colony size of 139,000 female flies was reached in mid-87. Fly reduction in the project area was achieved by means of IIS (with Deltamethrin, leaving a deposit of 150 mg/m^2) (Oladunmade *et al.*,1985)) in the boundary areas or by removal trapping with the Challier biconical trap. Both devices proved equally efficient in reducing native fly numbers: 97 - 99% reduction after 3 months with IIS, and 90% reduction after 2 months with the Challier trap, placed at intervals of 150-250 m. In both cases however, extending the period of control with traps and targets did not achieve eradication. A major concern was the loss of, on average, 30% of the IIS due to theft, flooding and fire (Takken *et al.*, 1986). The programme demonstrated that a ratio of 10 sterile to 1 wild male was a prerequisite to achieve eradication (a ratio of 3:1 only achieved control). It also showed, that different habitats (more humid

and more extensive riverine forest galleries as in Burkina Faso) and different species (*G. p. gambiensis*) demand different release rates and different time frames. Weekly releases for 18 months resulted in eradication in the pilot phase of 4 isolated forest patches (Takken *et al.*, 1986). The programme was extended over the entire 1,500 km^2 block and in 1988 the fly was declared eradicated (Oladunmade *et al.*, 1990).

These various pilot programmes amply demonstrated some of the advantages of the sterile male technique for use against tsetse flies:

1. Only the target insect is attacked and in those areas where the technique is used alone, it is environmentally friendly;
2. SIT is species specific and no development of resistance to the effects of sterile males is expected to develop;
3. SIT is most efficient at low population densities (insecticides are inefficient for low population densities) and, due to the low reproductive potential of tsetse, natural tsetse fly densities are usually low and require fewer sterile males than other insect species that are controlled similarly;
4. SIT can be used to prevent re-invasion and is cheaper per km^2 as compared with other (insecticide) applications.

The technique has however also some limitations:

1. The low reproductive potential of the tsetse fly makes the current systems of mass production cumbersome and expensive;
2. SIT is not efficient against high population densities and requires in most cases suppression usually by means of insecticides;
3. SIT has a delayed effect on the numbers of vectors present;
4. The release of large numbers of sterile males might lead to an increase of disease transmission at the beginning of the programme.

The use of the SIT against tsetse flies has proven to be successful where other conventional techniques have failed e.g. the eradication campaign from a total area of 200,000 km^2 in northern Nigeria by ground spraying could not be expanded southwards into the sub-humid and humid zone of the middle belt of Nigeria where application of ground spraying and aerial spraying became more difficult (see 4.2.). The SIT eradication programmes in the sub-humid zones of Burkina Faso and Nigeria, however, have demonstrated the feasibility of eradication

in these difficult habitats. Dame & Schmidt (1970) already predicted in 1970 that 'the sterile male technique will probably not be the ultimate panacea, but if the technique proves to be efficient and economic, it will add a very potent weapon to the control armoury'. The costs of the SIT programme against riverine species in Burkina Faso was compared with those of conventional programmes. The least costly of the methods (use of insecticide impregnated screens) became however as expensive as the SIT, if applied longer than 5 years (Brandl, 1988).

5.2. The concept of SIT

The concept of controlling, managing or eradicating economic important insect pests by affecting their reproductive capability was first conceived in the 1930s by an American scientist Dr. E.F. Knipling. During studies on the mating behaviour of screwworm flies (*Cochliomyia hominivorax)* in the laboratory, he observed that female flies tend to mate only once. Knipling speculated that if a way to sterilise the male flies could be found, these sterile males might be used to cope with large outbreaks of isolated screwworm infestations in the south-eastern part of the USA. A major breakthrough came with a publication of Muller (1950) showing that ionising radiation could induce sterility in insects without impairing their mating activity. Initial laboratory experiments in the 1950s showed that both male and female screwworm flies could be rendered sexually sterile by exposure to X - rays (Bushland & Hopkins, 1951) or gamma rays (Bushland & Hopkins, 1953) without excessive damage to their survival and competitiveness. There are 2 basic techniques to apply sterility in a pest population (Knipling, 1955, 1959): (1) mass-rearing of the target insect, sterilisation through radiation or exposure to chemical agents and the release in sustained numbers in the natural habitat large enough to outnumber the wild pest population and (2) the treatment of a portion of the natural population with a chemical that causes sterility rather than death (Knipling, 1969). In the first strategy, the overwhelming numbers of sterile males would limit the reproduction of the natural population in proportion to the ratio of sterile to fertile insects (with a 1:1 ratio and a 9:1 ratio, the reproductive capacity of the natural population is reduced with 50%, and 90% respectively). As a result, the densities of the natural insect population will decline. The effect of continued releases of sterile insects will however increase and finally culminate in a possible elimination of the target population. Theoretical models showing the trend of a natural insect population subjected to the release of sterile males are presented in Table 2, 3

Table 2. Theoretical model of a population decline when a constant number of sterile males are released among a natural population of 1 million females and 1 million males (after Knipling, 1955)

Generation	No. of virgin females	No. of sterile insects	Ratio of sterile to fertile insects	No. of insects reproducing
F1	1,000,000	2,000,000	2 : 1	333,333
F2	333,333	2,000,000	6 : 1	47,619
F3	47,619	2,000,000	42 : 1	1,107
F4	1,107	2,000,000	1,807 : 1	<1

Table 3. Trend of an insect population, increasing fivefold each generation, subjected to control by the sustained release of competitive sterile insects when 90% of the total population consists of sterile insects that have been released (after Knipling, 1955)

Generation	No. of fertile insects	No. of sterile insects	Ratio of sterile to fertile insects	No. of insects reproducing
Parent	1,000,000	9,000,000	9 : 1	100,000
F1	500,000	9,000,000	18 : 1	26,316
F2	131,580	9,000,000	68 : 1	1,907
F3	9,535	9,000,000	944 : 1	10
F4	50	9,000,000	180,000 : 1	0

Table 4. Trend of an insect population subjected to various types of control (after Knipling, 1964)

Generation	Normal trend fertile insects (5 x rate of increase)	Control by insecticides at 90% level	Control by insecticide treatment followed by sterile insect releases
Parent	1,000,000	1,000,000	1,000,000
F1	5,000,000	500,000	500,000
F2	25,000,000	250,000	250,000
F3	125,000,000	125,000	125,000
F4	125,000,000	62,500	62,500
F5	125,000,000	31,250	16,450
F6	125,000,000	15,635	1,190
F7	125,000,000	7,812	0

and 4 (Knipling, 1955, 1964). The first model (Table 2) assumes a stable insect population of 2 million insects (50% females) in equilibrium with the environment and subjected to a release of 2 million fully competitive sterile males. As the males are equally competitive, 75% of the native females would mate with a sterile male, resulting in only 333,333 fertile insects in the next generation. If the rate of releasing 2 million sterile male insects is continued, the second generation females will be outnumbered by a ratio of 6 to 1 giving a third generation of only 47,619 fertile females. Only 1,107 fertile females would remain in the next generation overwhelmed by a ratio of 1807 sterile to 1 fertile male. This theoretical model is based upon a insect population which replaces itself exactly each generation. Natural populations however decline or increase depending on complex interactions with the environment. Knipling (1964) assumed that a natural population can increase fivefold per generation in the absence of any control practice. Table 3 presents data of a natural insect population of 1 million insects, increasing fivefold with each generation, but subjected to an overflooding ratio of 9 sterile insects to 1 wild. In Table 4, a comparison is given of a natural insect population of 1 million insects, increasing five fold each generation (up to the assumed maximal density of 125 million when intraspecific competition becomes a limiting factor) and subjected to insecticide control and a programme combining insecticides for 3 generations followed by release of sterile males at a 9:1 ratio. In the first programme, 7,812 fertile insects still persist in the seventh generation whereas in the second programme, the population is by then eradicated (Knipling, 1964). These theoretical models clearly demonstrate the major advantage of the SIT method i.e. the control effort becomes more economical and efficient as the natural population declines and increasing ratios of sterile to wild males are achieved (Dame, 1970). This is in contrast with conventional methods of killing insects, where the continued use of the same treatment will result in the same percentage effect regardless of population density (LaChance *et al.*, 1967) and becomes therefore less efficient in terms of numbers killed as the natural population declines (Dame, 1970).

Sterility or the inability to produce offspring, can be caused by infertility in the females, inability to mate, aspermia or sperm inactivation in the males, and induction of dominant lethal mutations in the male or female reproductive cells. Sterility based upon dominant lethal mutations is the kind most successfully used in insect control programmes (LaChance *et al.*, 1967). A dominant lethal mutation is a nuclear change in the germ cells without affecting their maturation but

killing the zygote during its development (Van Borstel, 1962). Radiation induced dominant lethal mutations arise as a result of chromosome breaks in the gametes. During cell division, these chromosome fragments are lost because they lack a centromere which is responsible for inclusion in the daughter nucleus (LaChance *et al.,* 1967). This leads to chromosome imbalance with the formation of dicentric chromosomes and breakage-fusion-bridge cycles during cleavage divisions in the zygote. Death usually occurs during the early divisions before the formation of the blastoderm (LaChance *et al.,* 1967). Research conducted with the tsetse fly *G. p. palpalis* confirmed that in 95% of the eggs fertilised by sperm of irradiated males, embryogenesis was inhibited in the early cleavage division stages. This was then followed by lytic processes and the extrusion of the dead eggs between day 14 and 20 (Matolin & Van der Vloedt, 1982). In 5% of the eggs however, advanced stages of embryogenesis were reached despite treatment of sperm up to 140 Gy (Matolin & Soldan, 1982). The finding that the occurrence of malformed embryos increased in eggs expelled during later reproductive cycles, indicates the possibility of some repair to the genetic damage (Matolin & Soldan, 1982).

It needs to be stated that not all insects are suitable for an approach with SIT. Factors such as the extend of the economic damage caused by the insect pest, density of the natural insect population, costs involved in mass production of the insect and some crucial biological aspects (possibility of producing competitive insects) have to be determined (Knipling, 1969). Some prerequisites for the successful implementation of the SIT are:

a. Colonisation of the insect should be feasible and mass production possible at reasonable cost to provide the required number of sterile insects. Refusal to mate in captivity, inhibition of development of the immature stages under laboratory conditions, inadequate spatial requirements are only a few reasons limiting the colonisation of many insect species at present;

b. Insects reared in the laboratory and released into nature should be as competitive (refers to mating ability of the sterilised insects) as the wild insects. However, various conditions inherent to the artificial colonisation make released insects usually less competitive. In some occasions, the vigour of the reared insect is reduced due to stress experienced during rearing, handling, sterilisation and release. In addition, differences in behaviour, quality and distribution between wild and released insects can

result in the failure of a SIT programme. During releases of *Anopheles quadrimaculatus* in Florida, no evidence of mating with sterile males was found. Careful examination of the situation revealed a behavioural difference with preferential matings occurring between sterile males and sterile females and between wild males and wild females. Another example of behavioural incompatibility occurred during some Lepidoptera programmes i.e. sterile females would call the wild males at different times during the night than would wild females;

c. The female of the insect to be controlled must mate only once or if more frequent matings occur, the sperm of gamma treated males must be equally competitive with the fertile sperm;

d. The population density of the target insect must be inherently low or reduced by other means to make it economically feasible to obtain the desired sterile to wild male ratios;

e. A thorough knowledge of the biology of the insect under consideration i.e. dynamics of the population on a seasonal basis, distribution patterns, movement, migration flight range, mating behaviour, breeding areas, host preferences etc. (Lindquist, 1969). In many programmes, mobility of the target insect has been severely underestimated resulting in persistent migration from outside the control area;

f. The method must be applied to the whole population of the target species or that part of the population which can be isolated by natural or artificial barriers (e.g. the situation in Zanzibar) i.e. area wide control as opposed to field by field conventional control.

6. Rearing of tsetse flies

The tsetse fly is unique among the insects in that both sexes are obligatory blood feeders. They produce few (6-10) offspring during their life span, require specific optimal climatic conditions and are extremely susceptible to insecticides, making the rearing of the insect in large numbers very complex. It is therefore not surprising that the early attempts to maintain tsetse flies in the laboratory were not very successful. Small numbers of *G. m. submorsitans* (Roubaud, 1917) and *G p. palpalis* (Mellanby & Mellanby, 1937; Rodhain & Van Hoof, 1944) were

successfully kept under laboratory conditions for a few generations, but non of these colonies were self sustaining and relied on input from field collected flies. Geigy (1948) managed to sustain a colony of *G. f. quanzensis* for more than 5 years in the Swiss Tropical Institute, but the colony was again dependant on regular influx from field collected pupae. The first self sustaining colony (*G. morsitans*) was kept by Azevedo & Pinhão (1964) in Lisbon which reached a maximum colony size of 3,000 flies. Female flies were kept singly in a climatically controlled room at a temperature of 26°C, 70% R.H. and a 12 hour light cycle. Pupae were placed in tubes containing moist sand and eclosion rates of 98% were obtained. The flies were fed on the shaved flanks of guinea pigs.

Important contributions towards rearing of tsetse flies on a larger scale were made by the team of Nash & Jordan in Bristol, who established a colony of *G. austeni* from pupae collected on Zanzibar. Major innovations were: (1) flies were allowed to emerge from pupae placed on the surface of sand, (2) more than 2,500 pupae could be kept in one emergence cage (emergence rate of 98%), (3) flies were kept in groups of 10 in Geigy cages (6 x 3.4 x 2 in.) or singly in tubes for research purposes, (4) feeding was feasible on a variety of living host animals i.e. calves, goats and lob eared rabbits. It was however the feeding on the host animals which created most of the difficulties:

(1) feeding on calves: despite the high productivity of the *G. austeni* colony, the use of calves for feeding was not very practical in view of their seasonal availability, their rapid growth to unmanageable size and sanitation problems caused by their liquid faeces (Jordan *et al.*, 1966; Ward, 1970),

(2) goats were found to be much better suited for feeding but the skin became rapidly scaly and edemtous (Ward, 1970). This skin sensitisation could be prevented by slowly building up the goats resistance by gradually increasing the challenge of fly bites (Nash *et al.*, 1966a). Moreover, goats could only be used for feeding every third or fourth day with the best performance (in terms of survival, pupae production and pupal weight) of the female flies when fed on pregnant goats. This was attributed to an increased uptake of blood or to an unknown factor in the blood which increased its nutritional value (Nash *et al.*, 1966a). A maximum of 700 flies could be fed on a single goat without inflicting skin damage.

(3) the lob eared rabbit has likewise been used as a host, with fly survival (*G. austeni*) (91% on day 60), pupal production (11.4 pupae per female) and pupal weight significantly higher compared to goat

fed flies (Jordan *et al.*, 1967). The rearing of rabbits under tropical conditions is however rather difficult due to their susceptibility to diseases (e.g. pneumonia), reproduction problems at high relative humidity and nutritional difficulties during the dry season (Cuisance *et al.*, 1980b),

(4) the most suitable living host so far used is the guinea pig with a potential challenge of 150 flies (*G. p. palpalis*) per day when used every 2 nd day for feeding (Van der Vloedt, 1982).

Rearing of tsetse flies using the *in vivo* technology requires the maintenance of two production systems: the flies and the host animals. The maintenance of a host colony requires adequate facilities, sufficient labour and veterinary attendance, rendering the method extremely expensive (Laird, 1977). In the Nigeria SIT programme (see 4.5.1.1.), a host animal size of 1000 guinea pigs was required for a colony of 30,000 *G. p. palpalis* whereas the same amount of flies can now easily be maintained on 10-15 litres of processed blood per week using membrane feeding techniques (Feldmann, 1993). In addition, other problems with host animals like insecticide treatments before the animals are bought, skin problems due to frequent feeding (Nash *et al.*, 1966a), disease, antibiotics and hormone treatments might impede colony performance (Feldmann, 1993).

In the *in vitro* feeding technique, blood is offered to the flies under an artificial membrane. The membrane should exercise the same attraction for the flies as the host skin to ensure successful piercing of the membrane by the proboscis. Logically, skins of host animals were used in the first trials but only very limited numbers of flies could be fed, initiating the search for artificial membranes. The agar-agar membrane incorporated in terylene netting was a success but preparation of the membrane every day and bacterial proliferation remained serious drawbacks (Mews *et al.*, 1972; Bauer & Wetzel, 1975). This work culminated in the development of a silicone membrane (Bauer & Wetzel, 1976) which is currently still used in the Seibersdorf and Tanga production units. The membrane has several advantages: multiple use, can easily be cleaned in a washing machine and sterilised in a heat sterilising oven.

The blood, defibrinated by mechanical stirring, is collected from the slaughterhouse under clean and sterile conditions from disease free and healthy animals. To ensure adequate decontamination, the blood is irradiated with a gamma dose of 1,000 - 1,500 Gy. Each blood sample is rigorously tested for its biological quality by assessing the Packed Cell Volume (PCV), bacterial contamination and production parameters during a 25-day bio-assay (Feldmann, 1993). During the latter,

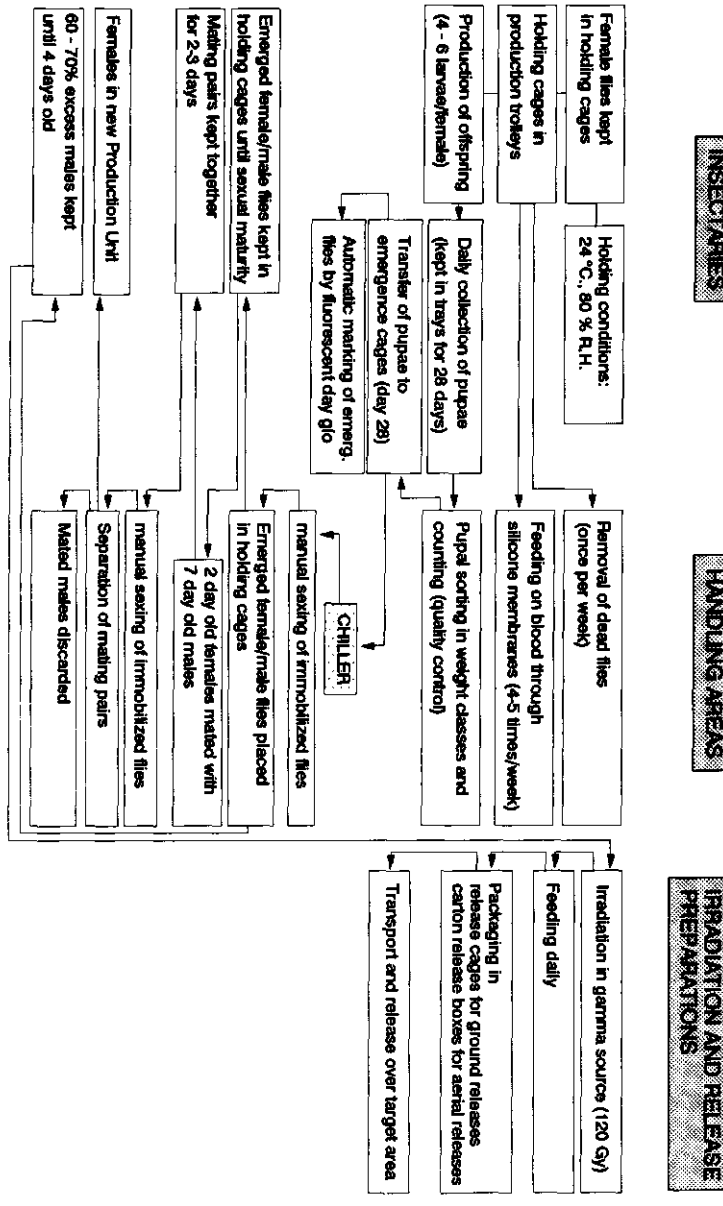

Fig. 7 Diagram of the mass rearing procedures of tsetse flies at the IAEA 's laboratory at Seibersdorf

production, essential biological parameters (survival, abortions, pupal weight) are assessed of a group of 30 female flies (Van der Vloedt, unpublished). Initially, pupae produced by female flies fed on the *in vitro system* were 10% lighter than those originating from *in vivo* colonies (Laird, 1977) indicating that these flies ingested less blood. To overcome this problem, phagostimulants like ATP are currently added to supplement the blood meal. At present, 7 tsetse species are completely adapted to *in vitro* mass rearing conditions and are maintained at the Seibersdorf laboratory (*G. austeni, G. p. palpalis, G. f. fuscipes, G. tachinoides, G. brevipalpis, G. pallidipes* and *G. morsitans submorsitans*).

The current rearing procedures, including methods for sterile male releases, used at the Entomology Unit in Seibersdorf and at the TTRI in Tanga are presented in Fig. 7. Pupae are collected daily, stored in petri-dishes (500/dish) and transferred to emergence cages 3-5 days before emergence. Sexing of the emerged flies is done manually at 4°C in fly chillers (modified deep freezers). 2-3 day old females (8 days old in the case of *G. pallidipes*) are mated in groups of 20 - 30 flies with sexually mature 6-7 day old males. A mating ratio of 1:1 is used in case no excess male material is required and is decreased to 1 male per 3 females during operational sterile male releases. Sexes are kept together for 2-3 days and then separated again manually in the chillers. Females enter the production units where their performance is closely monitored: survival of females in each unit is checked every week whereas pupae production and pupal weight as defined by its size are checked daily. Pupal period, percent emergence and sex ratio is monitored from a batch of 100 pupae. To be self sustaining, female flies should not be discarded before reaching the age of 80 days, daily mortality should not exceed 1%, more than 0.6 pupae should be produced per female per day and pupal emergence should be 85%. In addition, a stable colony produces a constant frequency distribution of the pupal weights. A shift towards lower weight classes immediately indicates a deviation from optimal rearing conditions (Van der Vloedt, 1982). The importance of the monitoring of the weight classes is demonstrated by the performance of the offspring i.e. females emerging from lower weight class pupae have a lower fecundity, higher mortality and produce offspring of lower weight (Van der Vloedt, 1982). It is estimated that a colony of 100,000 females, maintained for 18 weeks involves 140 man hours by experienced technicians (Feldmann, 1993).

In the past 30 years, enormous progress has been made in mass rearing of tsetse flies. The largest colonies have so far been maintained in Africa to support operational release programmes:

50,000 - 55,000 producing female *G. m. morsitans* in Tanga (Williamson *et al.*, 1983a), more than 100,000 *G. p. gambiensis* and *G. p. palpalis* in Burkina Faso and Nigeria (Politzar & Cuisance, 1984, Oladunmade *et al.*, 1990). Currently, a colony of 300,000 *G. austeni* is maintained at the TTRI, Tanga to supply the tsetse eradication project on Zanzibar the required amount of sterile males.

7. Objectives of the research

The presence of the tsetse fly in Africa is a seriously limiting factor for the development of the livestock sector on the continent and poses enormous threats to the health of the African people (ILRAD, 1993). Ever since the link between the tsetse fly and the disease (trypanosomiasis) was established (Bruce, 1895), many scientists have devoted their career to elucidate the biology of the fly, the complex interaction between vector, parasite and host animals and numerous attempts have been made to keep the tsetse scourge under control (Buxton, 1955; Mulligan, 1970). Of the various control methods, the Sterile Insect Technique might provide potential where other methods have failed (Takken *et al.*, 1986). Up to date, the SIT was applied in 3 integrated efforts for the eradication of tsetse (Tanzania, Burkina Faso and Nigeria) and a 4 th programme is currently being conducted on the island of Zanzibar, Tanzania (Williamson *et al.*, 1983 a,b,c,d; Politzar & Cuisance, 1984; Oladunmade *et al.*, 1990, Vreysen, 1992a, b). The concept of the SIT was only conceived fairly recently (Knipling, 1963), and despite the fact that much research has been conducted, many questions still remain unanswered, especially with respect to the effects of the irradiation treatment on the biology of the tsetse fly in general and the biological quality of the male and female flies for release in particular. With this study, it is attempted to fill in some of the gaps.

7.1 How and when to induce sterility?

The SIT concept relies on inducing sterility in one or both of the sexes. Different insect species require different doses of gamma radiation to induce 95-100% sterility. Whereas most Diptera are sterilised with doses under 100 Gy, the majority of Lepidoptera e.g. require doses between 200 and 500 Gy (LaChance *et al.*, 1967). The biological efficiency of a single organism as well as of a population is expressed by its fitness and the overall radiation response is measured by means of the fitness components, reproductive capacity, development rate and

adult life span (Nothel, 1968). As the SIT is species specific in its action, laboratory studies have to be conducted to determine the optimum radiation dose and the optimum stage for treatment in such a way that mating behaviour, competitiveness and longevity of the insects is not seriously affected. Detailed information on the effect of ionising radiation on the biological quality of the tsetse fly is available for some species: *G. p. gambiensis* (Taze *et al.*,1977), *G. p. palpalis* (Van der Vloedt *et al.*, 1978), *G. austeni* (Curtis, 1968; Vreysen *et al.*, 1992) and *G. m. morsitans* (Curtis & Langley, 1972; Langley *et al.*,1974). For other species, the obtained data are incomplete and obscured due to unknown age of the treated material (*G. pallidipes*) (Dean & Clements, 1969) or small sample size (*G. tachinoides*) (Itard, 1968). Dose response studies were carried out on 3 tsetse species of major economic importance in West and East Africa (*G. tachinoides, G fuscipes fuscipes* and *G. brevipalpis*). They are all potential candidates for eradication campaigns using a SIT component by virtue of their dense habitats. The results of this study are described in chapter 2. **The specific aim of this study on comparative gamma radiation sensitivity of *G. tachinoides, G. fuscipes fuscipes* and *G. brevipalpis* was to analyse the correlation between radiation dose applied in the adult stage and fertility, reproductive status and longevity.**

7.2 Are radiation treatments in the early pupal phase feasible?

High quality tsetse flies can also be obtained by giving the sterilising irradiation treatment to pupae, providing the treatment is administered during the last stages of their development (Dean & Wortham, 1969; Curtis & Langley, 1972.; Langley *et al.*, 1974; Williamson *et al.*, 1983b). Treating pupae with radiation in earlier stages has failed due to excessive somatic damage resulting in premature pupal death or as young adults (Van der Vloedt *et al.*, 1976). Moreover, as the entire process of spermatogenesis occurs in the pupal phase and the adult male emerges with its full potential of spermatozoids, special attention is required when irradiating tsetse flies as pupae (Itard, 1970). Females of some tsetse species are known to mate more than once in the laboratory (Jordan, 1958) and consequently, a mating with a sterile male with unviable sperm, is wasted if a second mating occurs with a fertile male with motile sperm. Chapters 3 to 5 are devoted to a study analysing the effects of various doses of gamma radiation on *G tachinoides* pupae during various stages of their development and to explore ways of obtaining high quality sterile males by radiation

procedures applied during earlier development stages. **The specific aims of the studies on pupal treatments of *G. tachinoides* were to analyse the effects of (1) different doses of gamma radiation administered during various phases of the pupal development, (2) irradiation of pupae in mid pupal phase under nitrogen atmosphere and splitting the radiation dose in fractions, and (3) chilling and irradiation during the mid pupal phase, on fertility, reproductive status and average longevity of the male and female flies.**

7.3 Is a combination of hybridisation and irradiation feasible for control?

One of the advantages of the SIT i.e. its species specificity, implies also a limitation: a multiple species approach requires the rearing of the various target species with subsequent higher costs involved. Using the hybridisation capacity of closely related sympatric and allopatric species or subspecies, the potential exists of rearing a limited number of tsetse species which can be deployed for eradication of a larger group of closely related species. Most of the research on hybridisation has been conducted on species of the *morsitans* group (Curtis, 1972; Gooding, 1984; Rawlings, 1985) with recently some studies including members of the *palpalis* group (Gouteux & Millet, 1984; Gooding, 1988). In this thesis, the combined use of induced sterility and hybridisation between two *palpalis* species is proposed. The cross breeding studies between *Glossina palpalis palpalis, Glossina palpalis gambiensis* and *Glossina fuscipes fuscipes* are described in chapters 8 - 9. **The specific aims of the study on hybridisation of closely related *palpalis* species were: (1) to analyse the mating behaviour and fertility of the interspecific crosses (*Gpp* and *Gpg*; *Gpp* and *Gff*), (2) fertility of the offspring (*Gpp* and *Gpg*), (3) mating preference in larger laboratory cages (*Gpp* and *Gpg*; *Gpp* and *Gff*) and (4) the effects of ionising radiation on mating behaviour, fertility and mating preference (*Gpp* and *Gpg*). The hybridisation study with *Gpp* and *Gpg* was complemented with a morphometrical analysis of the genital armature of the hybrids resulting from these crosses.**

7.4. The use of sterile female flies as a monitoring device

Each tsetse control programme requires a sound evaluation technique to

monitor its progress and finally its success. In SIT operations, monitoring schemes are usually developed and used that can detect the fluctuations in native population densities, the ratios of sterile to wild males and the impact of the matings on the reproductive systems of the female wild flies. Problems are encountered in those situations where the densities of the fly populations drop below the detectable level of the monitoring device e.g. when approaching the end of the eradication phase or due to the lack of an efficient trapping system for a particular species (e.g. *G. austeni* on Unguja island). This inability of trapping techniques to monitor low density insect populations imposes serious limitations on decision making in operational programmes. A zero catch does not necessarily mean that there are no more wild insects in the habitat. One option of increasing the efficiency of the trapping device is by the release of gamma sterilised females as was proposed by Van der Vloedt & Barnor (1984). Existing wild male flies will be exposed by spermathecae of the sterile female containing sperm. Little however is known about the effects of gamma radiation on the mating behaviour and reproductive capacity of female flies apart from initial studies by Dean & Wortham (1969), Dean & Clements (1969) and Van der Vloedt & Barnor (1984). Therefore, the reproductive implications of gamma radiation on female flies of two species of tsetse (*G. austeni* and *G tachinoides*) was investigated in detail (chapter 6 and 7). **The specific aim of the study on irradiation of female *G. austeni* and *G.tachinoides* was to investigate: (1) the dynamics of the follicle development in females after exposure to sterilising doses of gamma rays (*G. austeni*), (2) fecundity and status of the reproductive organs in relation to radiation dose and timing of treatment, (3) receptivity of treated and untreated females for mating with untreated males in laboratory cage experiments and (4) multiple mating behaviour of gamma treated female flies.**

7.5. A technique to distinguish mating between palpalis species

Evaluation of a release campaign based upon the high hybridisation capacity of *G. p. palpalis* and *G. f. fuscipes* might be complemented by analysis of the mating scar patterns on the female's abdomen. Slight morphological differences between the superior claspers of the males of the two species (Machado, 1954), result in slightly different mating scar patterns. **The specific aim of the mating scar study (chapter 12) of *G.p.palpalis* and *G.f.fuscipes* was to quantify biometrically their mating scar pattern.**

7.6. Efficiency of sticky panels for trapping G. austeni on Unguja island

Traditional survey techniques have failed completely in trapping *G austeni* in sufficient numbers to make a sound evaluation possible (Turner, 1984). Hall (1986) proposed to use panels made sticky with a non setting adhesive as an alternative to the traditional tsetse traps. This original idea was followed up by Schönefeld (1988) and Madubunyi (1990) who designed various models of sticky panels. A sound evaluation of the different types of sticky panels was however still lacking. Therefore, a contribution was made (described in chapter 13) to evaluate the sticky panels as a monitoring device for *G. austeni*. **The specific aims of the study on sticky panels were to evaluate their efficiency for trapping *G. austeni* and the composition of the fly samples in relation to panel shape, colour and used sticky material.**

7.7. Quality control of mass reared tsetse flies

The most important factor in any SIT programme, determining its success or failure, lies in the quality of the laboratory reared and released insects. This quality is affected or influenced by the vigour and productivity of the stock colony and the general procedures of colonisation, handling, sterilisation, transportation and release methods. Procedures to assess this quality are implemented in operational programmes in order to detect problems immediately before they become critical and launch emergency actions to rectify the situation. In chapter 14, quality control procedures, developed and applied in the operational SIT programme against *G. austeni* on Unguja island are described and an assessment is given of the biological quality of the flies in the laboratory, during the transport and after release in the natural habitat. **The specific aims of the study on sterile *G. austeni* fly quality control, was to assess the effects of colonisation, handling and release methods on the biological quality of the released *G. austeni* flies.**
In any insect control programme the real answers are to be found in the results of the field operations. Evaluation of data on the impact of the SIT method on Unguja are discussed in the final chapter (no. 15).

The experiments described in chapters 2 - 11 were conducted at the Entomology Unit of the IAEA's Laboratories in Seibersdorf, Austria. The work described in chapters 13 and 14 was carried out at the Tsetse and Trypanosomiasis Research Institute, Tanga (United Republic of

Tanzania) (Laboratory experiments) and at the Jozani forest and the Laboratories of the Department of Livestock Development on Unguja island (Zanzibar, U.R. of Tanzania).

Part 1 The effect of ionising radiation on the survival and reproductive biology of pupae and adult flies

Chapter 2

COMPARATIVE GAMMA RADIATION SENSITIVITY OF *Glossina tachinoides* Westwood, *Glossina fuscipes fuscipes* Newstead and *Glossina brevipalpis* Newstead (Diptera, Glossinidae)

Abstract

The effect of gamma radiation doses ranging between 10 and 180 Gy on 4-6 day old adult males of *Glossina tachinoides, Glossina fuscipes fuscipes* and *Glossina brevipalpis* was studied. Fecundity of their mates was reduced by 95% following exposure to 120 Gy, 80 - 100 Gy and 50 Gy of adult male *G. tachinoides, G. f.. fuscipes* and *G. brevipalpis* respectively. Insemination ability of the males and sperm motility were not adversely affected by the radiation treatment.

The higher proportion of dominant lethals in the sperm of the three species with increasing radiation doses was reflected in the reproductive status of the female mates i.e. an increasing percentage of females showing imbalances between intra-uterine content and ovarian development (females with an empty uterus due to expulsion of a dead embryo after embryonic arrest or a degenerating egg *in utero.*) and an acceleration in follicle development associated with successive unsuccessful cycles.

Viability of offspring fathered by *G. tachinoides* males was significantly reduced (except for males treated with 80 Gy). No reduction in emergence rate was observed for the offspring of treated *G. brevipalpis* and *G. f. fuscipes.* In the F_1 progeny of all treated groups, no significant bias towards one of the sexes was found.

The average life span of *G. tachinoides* and *G. f. fuscipes* males treated with doses of 80 Gy and higher and of *G. brevipalpis* males treated with doses exceeding 140 Gy was significantly reduced as compared with untreated controls. Male *G. brevipalpis* treated with doses ranging between 10 and 40 Gy however, showed a significant radiation induced increase in average life span.

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INTRODUCTION

Glossina tachinoides and *Glossina fuscipes fuscipes* are both important vectors of human and animal trypanosomiasis. They belong to the *palpalis* complex and inhabit riverine gallery forests in Western and Central Africa and the low-altitude valleys of the Ethiopian highlands (*G. tachinoides*). Species belonging to the *fusca* group are also potential vectors of trypanosomiasis but are considered to be of minor economic importance by virtue of their confinement to the tropical rain forests. *Glossina brevipalpis*, however, inhabits evergreen thicket associated with ground water and forest islands in the savannah (Ford, 1971) and can consequently come into close contact with domestic animals. Occasionally it feeds on cattle (Weitz, 1963) and its potential as a vector for *Trypanosoma vivax* and *T. congolense* was demonstrated by Harley (1965, 1966), Moloo *et al.* (1988) and Moloo (1992).

The use of the Sterile Insect Technique to control and eradicate tsetse fly population requires the mass rearing and sequential releases of large quantities of competitive sterile insects (Knipling, 1963). The last two decades, most of the economic important tsetse species have been adapted to mass rearing using both *in vivo* and *in vitro* techniques (Nash *et al.*, 1971; Mews *et al.*, 1976; Bauer *et al.*, 1984; Itard & Bauer, 1984; Van der Vloedt, 1982; Van der Vloedt *et al.*,1987; Feldmann *et al.*, 1992). Since the early work of Potts (1958), Dean & Wortham (1969) and Dean & Clements (1969) showed that gamma radiation doses ranging from 80 to 160 Gy administered to *Glossina morsitans* pupae and *Glossina pallidipes* adults induced 80 - 95% sterility, the effect of gamma radiation on reproduction and fitness of the several other tsetse species has been investigated. Itard (1968) obtained complete sterility in male *G. tachinoides* by exposing 1-9 day old adults to 150 - 170 Gy gamma radiation and Offori & Czock (1975) found that a dose of 120 Gy was sufficient to induce 97% sterility. The same dose reduced fertility by 95% in male *Glossina austeni* (Curtis, 1968), *Glossina palpalis gambiensis* (Taze *et al.*, 1977) and *Glossina palpalis palpalis* (Van der Vloedt *et al.*, 1978) without altering viability, mating effectiveness and longevity. Langley *et al.* (1974) demonstrated the beneficial effect of nitrogen during irradiation of *G. morsitans morsitans* pupae in the late stage of development.

All three tsetse species are potential candidates for eradication programmes using the sterile insect technique in view of their dense habitats. No information on radiosensitivity is currently available for *G. fuscipes fuscipes* and *G. brevipalpis* and a more detailed analysis is

required for *G. tachinoides*. Therefore, a comparative study was undertaken to examine the effect of increasing radiation doses on fertility and viability of *G. tachinoides*, *G. f. fuscipes* and *G. brevipalpis* males.

MATERIALS AND METHODS

Experimental flies

All flies used for the experiments were derived from the stock colonies maintained on membrane feeding systems at the Entomology Unit of the IAEA's laboratories in Seibersdorf, Vienna. *Glossina brevipalpis* (Kenya strain) and *G. f. fuscipes* (Central African Republic strain) flies were kept in the same insectary at 23° ± 1°C and at a Relative Humidity of 85% ± 5%. Holding conditions for *G. tachinoides* (Burkina Faso origin) differed slightly with R.H. of 78% ± 5%. All flies were fed 6 times a week on equal proportions of frozen and thawed bovine and porcine blood (Wetzel & Luger, 1978; Van der Vloedt, *et al.*, 1987).

Radiation procedures and experimental design

A ^{60}Co Gammacell 200 providing a dose rate of ca. 135 Gy/min was used for all treatments. Males were given an irradiation treatment of 40, 80, 100, 120, 140, 160 and 180 Gy on day 4 - 6 following emergence. The experiment was replicated for *G. brevipalpis* with doses ranging from 10 to 80 Gy given in steps of 10 Gy. On day 8- 10 following emergence, males were mated in groups of 10 (*G. brevipalpis)* and 20 (*G. tachinoides* and *G. f. fuscipes*) at a 1:1 ratio with 2-3 day old virgin females. Males and females were kept together for 2-4 days in standard colony cages (diameter 11 cm x 4.5 cm height) and after separation pooled in larger holding cages (diameter 20 cm, height 4.5 cm) in groups of 60, 65 and 80 females for *G. brevipalpis*, *G. f. fuscipes* and *G tachinoides* respectively. Their survival and productivity was checked daily for 45 days (*G. f. fuscipes)*, 55 days (*G. tachinoides)* and 55-60 days (*G. brevipalpis)*. Larviposition receptacles were checked for expelled eggs and immature larval stages every 5-7 days starting on day 15 following emergence. In addition, all pupae were weighed on the day of deposition. At the end of the experimental period, all females were dissected and their reproductive status assessed. Motility of

91.7%, 89.1% and 90.0% of the pupae produced by untreated *G. tachinoides*, *G. f. fuscipes* and *G. brevipalpis* respectively emerged with 52.7%, 46.7% and 53.9% being females. Pupae fathered by 40 Gy (chi square = 10.8, $p < 0.01$), 100 Gy (chi square = 33.27, $p < 0.01$) and 120 Gy (chi square = 6.10, $p < 0.05$) treated *G. tachinoides* males showed a significant reduction in viability as compared to the control group with emergence rates of 82.7%, 55% and 66.6% respectively. All pupae fathered by irradiated *G. f. fuscipes* and *G. brevipalpis* males showed emergence rates comparable with the control group ($p > 0.05$). (The significant decrease in emergence rate (45.9%, chi square = 286.84, $p < 0.01$) observed for *G. brevipalpis* pupae produced by females mated with 10 Gy treated males seems aberrant and might have been caused by unexplained factors).

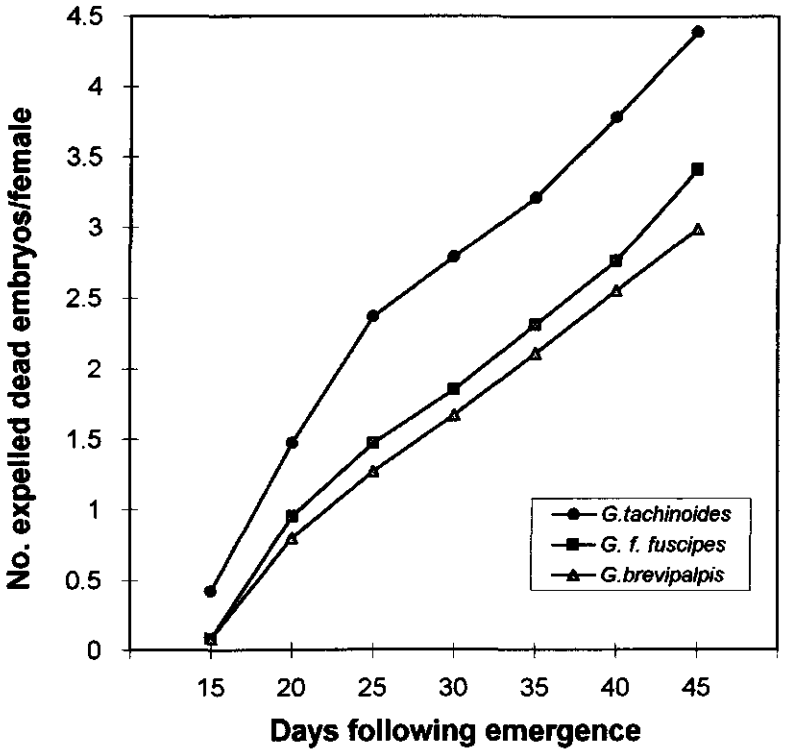

Fig. 1 Cumulative number of dead embryos recovered per female after indicated days following emergence. *G. tachinoides*, *G. f. fuscipes* and *G. brevipalpis* were mated with males treated with 120 Gy, 80 Gy and 50 Gy respectively.

Although more males flies emerged from pupae fathered by all treated *G. tachinoides* males and most of the treated *G. f. fuscipes* and *G. brevipalpis* males, the differences were revealed as non-significant ($p > 0.05$).

Reproductive status of females inseminated by irradiated males.

The dose dependent increase in sterility with increasing radiation doses of the males was reflected in the actual pregnancy stages of the females when dissected at the end of the experimental period (Table 2). In *G. tachinoides*, 43.3% and 46.6% of the control females were found with a recently ovulated egg and with a viable larva *in utero* respectively. 6.6% of the females displayed an empty uterus due to recent deposition of the third instar larva. A similar pregnancy picture was found for the other two species with 87.3% of *G. f. fuscipes* and 94% of *G. brevipalpis* control females with a recently ovulated egg or a viable instar larva *in utero*. A gradual increase in aberrations in ovary/uterus developmental stages was found with females inseminated by males treated with increasing radiation doses i.e. for the three species examined, the proportion of females with a viable larval stage *in utero* decreased and the proportion of females with an egg *in utero* showing evidence of embryonic arrest or an empty uterus caused by expulsion of the dead embryo increased. Almost no viable larvae were found in *G. tachinoides* and *G. f. fuscipes* females mated with males treated above 80 Gy and only 4.0% and 3.6% of *G. brevipalpis* females mated with 40 Gy and 50 Gy treated males were found with an advanced stage *in utero*. Moreover, 78.7%, 74.5% and 73.2% of *G. tachinoides*, *G. f. fuscipes* and *G. brevipalpis* females mated with males irradiated at the sterilising dose of 120 Gy, 80 Gy and 50 Gy respectively showed an uterus with a degenerating egg or an empty uterus due to abortion. In addition, more females in the treatment groups had blocked ovaries, possibly caused by prolonged retention of a degenerating egg *in utero* preventing ovulation.

The pronounced acceleration effect in follicle development due to successive unsuccessful cycles has been described for *G. austeni* (Curtis, 1968), *G. p. gambiensis* (Taze *et al.*, 1977) and *G. p. palpalis* (Matolin & Van der Vloedt, 1982). Our data for the three species examined showed that the higher number of dead embryos recovered in the treatment groups, could indeed be correlated with the advanced configuration stages of the females (Table 2). In addition, females

Table 2. Reproductive status of female *G.tachinoides*, *G.f.fuscipes* and *G.brevipalpis* inseminated by untreated and gamma irradiated males

Species	Dose (Gy)	F.N.O.S. (%) [1]					UTERUS CONTENT (%)						
		A_2	C_2	B_2	A_{1-2}	BL	Recently ovulated egg	Degenerating egg	Empty due to AB & BL [2]	Empty due to PL [3]	Viable Instar Larva I	Viable Instar Larva II	Viable Instar Larva III
G. tachinoides	Control	7.1	18.6	62.5	1.7	0.0	43.3	0.0	1.6	6.6	18.3	23.3	5.0
	40	4.0	12.0	60.0	18.0	6.0	21.1	11.5	26.9	9.6	1.8	13.5	7.7
	80	1.9	1.9	28.3	66.0	1.9	43.6	16.3	23.6	10.9	0.0	1.8	1.8
	100	1.6	1.6	28.6	60.3	7.9	16.9	38.5	29.2	12.3	0.0	0.0	0.0
	120	6.2	4.6	10.7	76.9	1.5	13.6	34.8	43.9	4.5	0.0	1.5	0.0
	140	0.0	1.7	8.3	90.0	0.0	6.5	48.4	38.7	4.8	0.0	0.0	0.0
	160	3.0	6.1	7.6	81.8	1.5	23.9	23.9	45.0	7.0	0.0	0.0	0.0
	180	6.8	13.5	5.1	71.2	3.4	9.8	41.0	49.2	0.0	0.0	0.0	0.0
G.f. fuscipes	Control	64.8	1.9	0.0	33.3	0.0	52.7	0.0	1.8	10.9	1.8	16.4	16.4
	40	66.0	28.0	0.0	2.0	4.0	21.5	19.6	33.3	9.8	2.0	13.7	0.0
	80	51.3	48.7	0.0	0.0	0.0	19.1	42.6	31.9	0.0	2.1	2.1	2.1
	100	45.5	52.7	1.8	0.0	0.0	21.1	38.6	33.3	5.3	0.0	1.8	0.0
	120	34.5	50.9	3.5	1.8	9.1	17.2	29.3	53.4	0.0	0.0	0.0	0.0
	140	40.5	45.2	0.0	0.0	14.3	10.9	37.0	52.2	0.0	0.0	0.0	0.0
	160	26.5	61.2	0.0	4.1	8.2	15.1	52.8	32.1	0.0	0.0	0.0	0.0
	180	38.6	52.6	0.0	3.5	5.3	20.0	43.3	35.0	0.0	1.7	0.0	0.0
G. brevipalpis	Control	11.4	63.3	17.7	6.3	1.3	22.6	0.0	3.6	2.4	7.1	23.8	40.5
	10	18.6	58.1	16.3	4.7	2.3	26.1	10.9	13.1	0.0	10.9	19.6	19.6
	20	18.0	40.0	34.0	6.0	2.0	35.8	15.2	7.6	3.8	9.4	18.9	9.4
	30	22.7	25.0	45.5	2.3	4.5	21.3	27.7	38.3	2.1	2.1	6.4	2.1
	40	2.1	27.7	55.3	10.6	4.3	16.3	24.5	49.0	6.1	2.0	2.0	0.0
	50	7.4	38.9	35.2	7.4	11.1	21.4	16.1	57.1	1.8	0.0	0.0	3.6
	60	3.8	30.8	53.8	3.8	7.8	22.2	27.8	48.1	0.0	0.0	1.9	0.0
	70	11.9	40.5	38.1	2.4	7.1	14.0	34.9	51.1	0.0	0.0	0.0	0.0
	80	4.3	21.3	63.8	4.3	6.3	21.3	25.5	53.2	0.0	0.0	0.0	0.0

1. Follicle next in ovulation sequence A,C,B,D.
2. Abortion and Blockage
3. Post larviposition

mated with treated males were characterised by an excessive building up of fat bodies.

Survival of untreated and treated males

The average life span of untreated and treated males of the three species is presented in Fig. 2 and 3. The average life spans of untreated *G. tachinoides* and untreated *G. f. fuscipes* males were similar with on average 57.6 ± 29.4 days and 57.5 ± 24.5 days recorded respectively. Treating males of both species with doses of 80 Gy or higher caused a significant reduction in their survival (*t* - test, $p < 0.05$). Average longevity was decreased with 11.7 and 13.2 days, 20.1 and 9.9 days and of 34.3 and 16.7 days for *G. tachinoides* and *G. f. fuscipes* males treated with 80 Gy, 120 Gy and 160 Gy respectively as compared with untreated males. However, LT_{50} (time lapsing to obtain 50% mortality) of 44 days, 42 days and 39 days, and 43 days, 50 days and 49 days were recorded for male *G. tachinoides* and *G. f. fuscipes* treated with 80 Gy, 100 Gy and 120 Gy respectively.

The observations with *G. brevipalpis* males were different. Untreated *G. brevipalpis* males survived significantly longer (average longevity of 73.9 ± 42.6 days) as compared with untreated *G. tachinoides* and *G. f. fuscipes* males ($p < 0.05$). In addition, male *G. brevipalpis* treated with low doses of 10 Gy, 30 Gy and 40 Gy showed a significant increase in longevity with average life spans of 110.1 ± 35.1 days, 106.2 ± 25.2 days and 107.1 ± 25.1 days respectively ($p < 0.05$). No significant differences were observed for males treated with doses ranging from 50 Gy to 140 Gy as compared with control males and high doses of 160 Gy and 180 Gy were required to decrease the life span significantly with 23.2 and 29.9 days respectively.

DISCUSSION

As expected, the proportion of dominant lethals induced in the sperm of male *G. tachinoides*, *G. f. fuscipes* and *G. brevipalpis* by gamma radiation increased with increasing doses. A dose of 120 Gy, 80 - 100 Gy and 50 Gy administered to 4 - 6 day old male *G. tachinoides*, *G. f. fuscipes* and *G. brevipalpis* respectively induced 95% sterility in the three species. Radiation sensitivity of male tsetse flies (Dean & Wortham, 1969; Dean & Clements, 1969; Taze *et al.*, 1977; Van der Vloedt *et al.*, 1978; Offori & Czock, 1975; Itard, 1968; Curtis & Langley, 1972) is lower than that

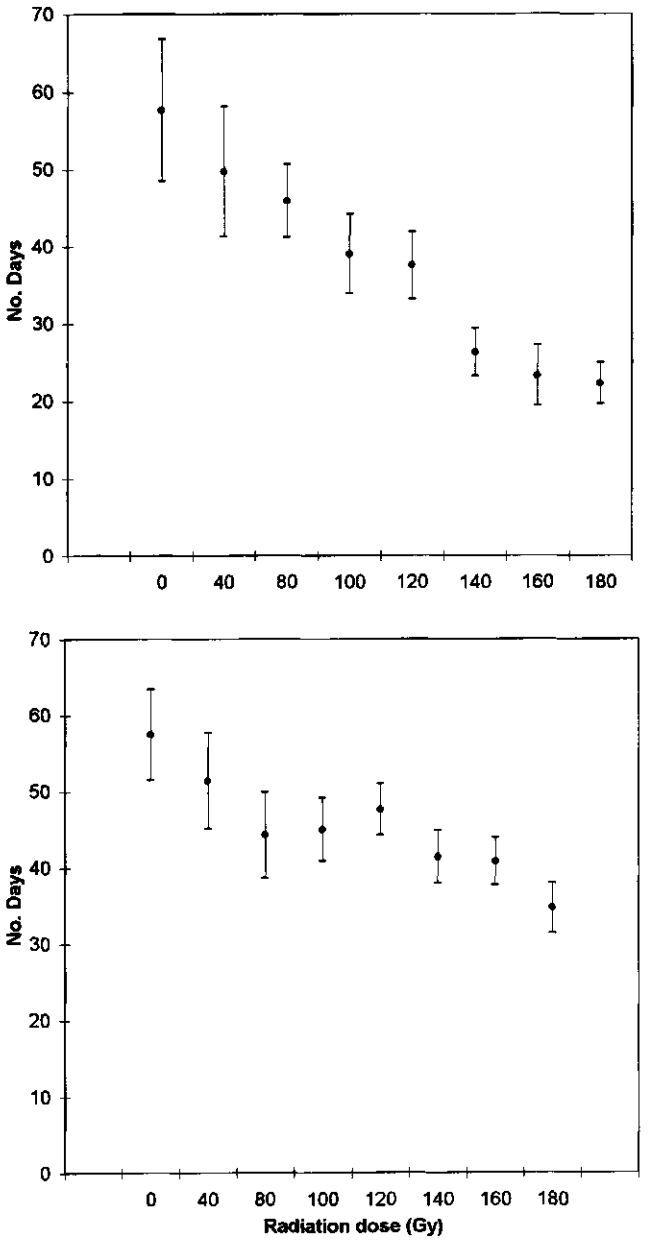

Fig. 2 Average life span of untreated and irradiated male *Glossina tachinoides* (top graph) and *Glossina fuscipes fuscipes* (bottom graph). The vertical bars denote 95% confidence limits.

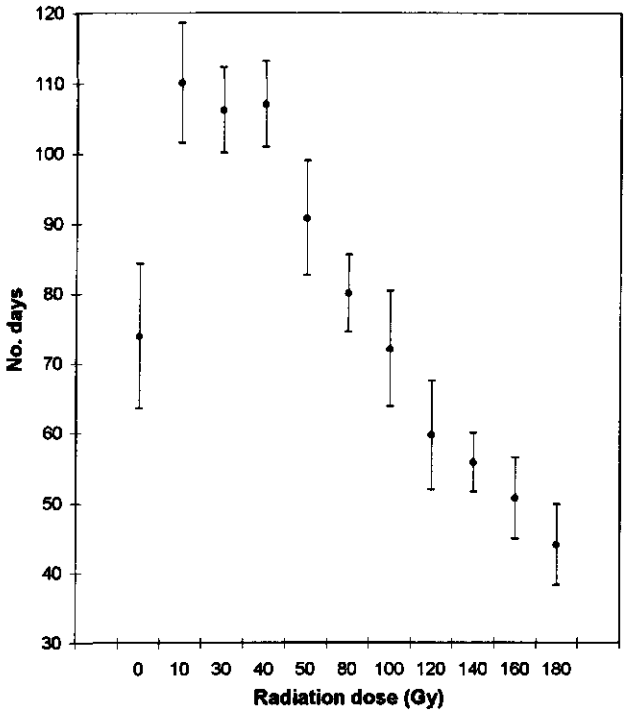

Fig. 3 Average life span of untreated and irradiated male *Glossina brevipalpis*. The vertical bars denote 95% confidence limits.

of most Diptera i.e doses above 100 Gy were required to achieve 95% dominant lethal mutations in the sperm of *palpalis* and *morsitans* species so far tested, whereas doses of 50 - 70 Gy are sufficient to sterilise adult male *Musca domestica*, *Hippelatus pusio*, *Cochliomya hominivorax* and *Aedes aegypti* (LaChance *et al.*, 1967). Our data on the levels of sterility of *G. f. fuscipes* and *G. tachinoides* males after treatment with 80 - 120 Gy in air are similar to those found for other species. The reason why the sperm of male *G. brevipalpis* is more susceptible to radiation remains unclear but is most likely chromosome related. Although *G. brevipalpis* has a higher number of chromosomes (2n = 16) (Maudlin, 1970) than *palpalis* and *morsitans* species (Gooding, 1984), other factors as the size of the chromosomes and location of the centromere might also be associated with the variation in radiosensitivity (LaChance *et al.*, 1967).

The induction of dominant lethal mutations in 50 to 120 Gy treated sperm of male *G. tachinoides, G.f. fuscipes* and *G. brevipalpis* was

expressed in the female mates by a high rate of extruded dead embryos. In addition, examination of the reproductive status of females of the three species, revealed an almost complete absence of immature larvae *in utero*. These findings indicate that the sterilising dose inhibits development in the three species during early or advanced stages of embryogenesis and are in accordance with the effect of ionising radiation on *G. austeni* and *G. p. palpalis* (Curtis, 1968; Matolin & Soldan, 1982; Matolin & Van der Vloedt, 1982). However, interspecific differences in the timing of the extrusion of the dead embryos became apparent. As was observed in females of *G. p. palpalis* (Matolin & Van der Vloedt, 1982), the first sterile egg in the majority of *G. f. fuscipes* and *G. brevipalpis* females was expelled between day 15 and day 20. The fact that 42% of the *G. tachinoides* females expelled the first sterile egg before day 15 can partially be attributed to an earlier maturation of the first follicle as compared to *G. brevipalpis* (Vreysen, unpublished data). However, other phenomena like interspecific differences in the phase when embryonic arrest occurs or a prolonged period of retention of the egg after cessation of the development might likewise be important.

The somatic damage caused by the irradiation of adult males is expressed by a reduction in average longevity compared with untreated males. Although life expectancy is significantly reduced for *G tachinoides* and *G. f. fuscipes* males irradiated with the sterilising dose of 120 Gy and 80 - 100 Gy respectively as compared to untreated males (57 days for both species), average longevity remains above 35 days and is comparable with data obtained for 120 Gy treated *G. austeni* males (Vreysen *et al.*, 1992). The radiation induced increase in average life span observed for *G. brevipalpis* males treated with doses of 10 to 40 Gy is a phenomenon observed in many insects, including several *Glossina* species. Dean & Wortham (1969) observed an increase in average longevity of *G. morsitans* males treated in the pupal stage with doses ranging from 10 to 20 Gy and 120 to 150 Gy. Likewise, an increase of male mean longevity was found for pupae irradiated in nitrogen with 70 Gy in the late pupal phase (Curtis & Langley, 1972). These observations contrast with our data of *G. f. fuscipes* and *G tachinoides.* In addition, the somatic damage caused by treating pupae with 120 - 150 Gy in air seems substantial and Dean and Wortham's observations cannot be explained by our present results. This radiation induced increase in survival of Diptera species has been attributed to several phenomena as (i) the apparent absence in this group of somatic cell renewal (Ducoff, 1972), (ii) a significant elimination of contaminating micro-organisms (Atlan *et al.*, 1970), and (iii) the fact

that certain physiological processes are slowed down by the radiation and/or that repair mechanisms in the insects are stimulated sufficiently by the slight destruction of certain tissues incurred by the radiation (Cork, 1957). However, the observed interspecific differences in average longevity of the three *Glossina* species studied might be correlated with species specific activity patterns. Although no assessment of fly activity was carried out, male *G. brevipalpis*, a more docile fly as compared with the more agile *G. tachinoides* and *G. f. fuscipes*, possibly became more lethargic due to the low dose gamma radiation, resulting in a decrease in expenditure of energy (Sullivan & Grosch, 1953).

It would be worthwhile to extend this study and examine the amount of induced dominant lethality in F_1 progeny of males irradiated with doses below that required for 100% dominant lethality. Curtis *et al.* (1973) have postulated that F_1 semi-sterility (50% sterility in a large proportion of *G. morsitans* F_1 individuals) combined with a sex ratio distortion towards males, would considerably reduce the requirements of sterile males in a release programme.

In conclusion, it is recommended that a radiation dose of 50 Gy, 80 Gy and 120 Gy be used for sterilising males of *G. brevipalpis, G. f. fuscipes* and *G. tachinoides* respectively, for use in an eradication programme with a SIT component.

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Chapter 3

RADIATION STERILISATION OF *Glossina tachinoides* Westwood PUPAE: I. THE EFFECT OF GAMMA RADIATION ON FEMALE AND MALE PUPAE OF DIFFERENT AGE

Abstract

The effect of gamma radiation on female and male *Glossina tachinoides* Westwood pupae of different age was determined following exposure to ^{60}Co in air at dose rates ranging from 10 to 120 Gy. The development process of male and female pupae was not affected when treated on day 25 - 28 post larviposition with doses up to 120 Gy. Radiation doses exceeding 40 and 80 Gy administered to 15 and 20 day old pupae reduced the eclosion rate of male flies indicating their higher susceptibility at younger stages to the radiation treatments as compared to females.

Gamma radiation treatment of 25 - 28 day old pupae resulted in higher levels of sterility as compared with sterility levels of males treated as adults. Survival of the males decreased with increasing doses and with decreasing pupal age at the time of treatment. Males treated as 28, 25 and 20 day old pupae with doses of 100 - 120, 80 and 20 Gy respectively, revealed a residual fertility of less than 5% with an average longevity exceeding 21 days. Moreover, their mating behaviour and insemination capacity were not adversely affected by the radiation treatment. Aberrations in the reproductive status of their female mates were similar to the ones found with males irradiated as adults. Treatment of 10 - 15 day old male *G tachinoides* pupae resulted in high pupal death, low survival rates during the first week following eclosion and reduced insemination capacity. Irradiation of *G tachinoides* pupae in air without an adverse effect on the flies is therefore limited to the last 10 days of the pupal development.

Doses up to 120 Gy given to 20 - 28 day old pupae, did not adversely affect the mating response and survival of female flies with a dose of 40 Gy being sufficient to induce complete sterility.

Table 1. Emergence rate of male and female *G. tachinoides* pupae irradiated with 10 - 120 Gy in air during various phases of their development

Irradiation day [1]	Dose (Gy)	Puparia no.	Adult emergence (%)			Content non-emerged pupae (no.)			
			total	females	males	female	male	lysis	early stage
Control	0	492	88.0	53.8	46.2	9	14	33	3
28	10	94	88.3	54.2	45.8	1	2	7	1
	20	99	88.9	51.1	48.9	1	5	2	3
	40	97	83.5	59.3	40.7	10	1	3	2
	80	100	83.0	59.0	41.0	3	5	5	4
	100	97	91.8	46.1	53.9	2	2	2	2
	120	98	83.7	43.9	56.1	4	6	5	1
25	10	100	93.0	61.3	38.7	1	3	3	0
	20	101	88.1	47.2	52.8	1	4	5	2
	40	100	87.0	57.5	42.5	6	3	4	0
	80	100	84.0	56.0	44.0	4	2	10	0
	100	97	82.5	58.8	41.3	5	1	10	1
	120	98	93.9	48.9	51.1	3	2	1	0
20	10	97	92.8	56.7	43.3	2	2	3	0
	20	100	93.0	45.2	54.8	2	2	2	1
	40	100	92.0	54.3	45.7	0	1	7	0
	80	100	81.0	49.4	50.6	2	3	5	4
	100	100	64.0	57.8	42.2	2	16	9	1
	120	99	54.5	83.3	16.7	4	25	3	0
15	10	100	83.0	60.2	39.8	3	3	9	2
	20	101	84.2	61.2	38.8	3	5	7	1
	40	99	80.8	56.3	43.8	2	10	6	1
	80	100	52.0	94.2	5.8	5	37	5	1
	100	100	39.0	100	0.0	9	47	5	0
	120	100	34.0	100	0.0	13	49	4	0
10	10	99	76.8	42.1	57.9	3	11	7	2
	20	97	2.1	0.0	0.0	54	36	4	1
	40	101	2.0	0.0	0.0	51	42	3	3
	80	100	0.0	0.0	0.0	56	36	5	0
	100	100	0.0	0.0	0.0	55	40	5	1
	120	101	0.0	0.0	0.0	53	43	4	1

1. Days post larviposition

INTRODUCTION

Sterilising tsetse flies by ionising radiation in the adult stage has been extensively studied and successfully applied in large scale eradication campaigns against riverine *palpalis* species using the Sterile Insect Technique in Burkina Faso and Nigeria (Politzar & Cuisance, 1984; Oladunmade *et al.*, 1990). In both campaigns, adequate rearing techniques and male handling schemes resulted in the production, transport and release of highly competitive adult male tsetse flies with an excellent survival and dispersal in the field (Politzar *et al.*, 1979; Bourne, 1982). This was different for species belonging to the *morsitans* group since laboratory reared *Glossina morsitans orientalis* males were found to be physiologically inferior due to underdeveloped flight muscles (Dame *et al.*,1968). Therefore, in a pilot trial in Mkwaja Ranch, Tanzania for the control of *G. m. morsitans*, male flies were sterilised in the late pupal phase and released as pupae in the field (Williamson *et al.*, 1983 b,d).

Although sterilising male tsetse flies in the late pupal stage has several advantages i.e. pupae are easier to handle, are less susceptible to damage and claim less space than adult males (Van der Vloedt *et al.*, 1976), a major concern remains the vectorial capacity of males released as pupae. Adult males, before being released, can't be given a blood meal which renders them less susceptible to trypanosome infections (Wijers, 1958; Gingrich *et al.*, 1982). In addition, it requires the separation of the female and male sexes. Although female and male eclosion is on average separated by two days, only 40% of the females can usually be selected before the onset of the male emergence flush. On the other hand, male emergence can be controlled by cooling without adversely affecting the quality of the male flies (Curtis & Langley; 1972; Langley *et al.*,1974).

Any tsetse control programme containing a SIT component, requires the maintenance of a stock colony of the target species large enough to support sequential releases of sufficient numbers of sterilised male flies. Critics of the technology have always focused on the high initial investment costs for buildings, training of personnel and the long preparatory phase. The technology can become more cost efficient by limiting the number of specialised mass production centres over the African continent. Each unit would mass produce the most important species and be in a position to serve large regional areas both in East and West Africa. This however, implies the transport of several species of tsetse pupae over long distances from the mass rearing unit to the

Table 2. Fertility of untreated female *G.tachinoides* mated with males irradiated with doses of 10 - 120 Gy during various phases of their pupal development

Irradiation day / dose [1] (Gy)	Initial females no.	Female survival day 45 % [2]	Mating status MS + / SP + % [3]	Mean puparial weight (mg) ± SD	Fecundity [4]	Production relative to control	No. aborted eggs	Emergence/ females %
Control	80	88.3	100 / 94.9	17.7 ± 2.8	0.089	100	25	85.2 / 54.3
28 / 10	20	98.9	100 / 100	18.1 ± 2.7	0.061	69.3	14	93.5 / 69.0
20	20	97.3	100 / 88.8	18.7 ± 3.1	0.046	51.8	40	90.0 / 61.1
40	20	100	100 / 100	16.7 ± 3.2	0.021	23.4	49	75.0 / 44.4
80	20	81.0	100 / 100	15.9 ± 2.5	0.008	8.8	74	3 / 4 / 0 / 3
100	20	100	100 / 100	—	0.000	0.0	83	— / —
120	20	92.3	100 / 100	—	0.000	0.0	63	— / —
25 / 10	20	91.5	100 / 88.8	16.0 ± 3.0	0.039	44.1	21	87.5 / 100
20	20	96.9	100 / 82.3	15.8 ± 4.9	0.039	44.6	30	57.1 / 37.5
40	20	99.4	94.7 / 94.7	17.0 ± 1.5	0.014	15.8	62	100 / 66.7
80	20	97.5	94.7 / 89.8	—	0.000	0.0	67	— / —
100	21	100	100 / 80.9	—	0.000	0.0	88	— / —
120	10	100	100 / 70.0	—	0.000	0.0	43	— / —
20 / 10	40	97.2	100 / 100	16.4 ± 3.0	0.041	46.2	71	76.9 / 36.7
20	31	98.2	100 / 100	12.6 ± 1.7	0.005	5.9	91	3 / 4 / 1 / 2
15 / 10	20	100	100 / 100	15.2 ± 3.2	0.017	19.1	67	8 / 9 / 3 / 8
10 / 10	14	92.9	100 / 61.5	—	0.000	0.0	59	— / —

1. Days post larviposition
2. Survival relative to mature female days
3. MS +: Mating Scars present
   SP +: Spermathecae impregnated with motile sperm
4. No. pupae per mature female day

target area or country. Extreme safe transport conditions can be obtained by shipping sterilised pupae. Moreover, recipient countries could then easily incorporate the SIT technology in their national tsetse control efforts even when the expensive radiation source is lacking.

This paper is the first in a series that reports on studies on the effect of ionising radiation on *G. tachinoides* pupae with special emphasis on the mid pupal phase. The aim of the study presented here was to analyse the relationship between radiation dose, phase of development of *Glossina tachinoides* pupae, fertility and adult survival and consequently identify the earliest treatment age which still gives optimum sterility and survival of the male flies.

MATERIAL AND METHODS

Pupae and fly material

All pupae used for the experiments were derived from the *Glossina tachinoides* colony, originating from the Central African Republic, and maintained on a membrane feeding system at the Entomology Unit of the IAEA 's laboratory in Seibersdorf, Austria. Pupae and adult flies were kept under normal colony conditions i.e. 23° ± 1°C and 75% ± 5% Relative Humidity. All flies were fed daily (except on Sundays) on equal proportions of frozen and thawed bovine and porcine blood (Wetzel & Luger, 1978).

Radiation procedures and experimental design

Batches of 100 puparia, collected on the same day and kept in plastic petri-dishes (diameter 5.5 cm, height 1.5 cm), were irradiated in air in a ^{60}Co source (dose rate of 6 - 7 Gy/min.) at doses ranging from 10 to 120 Gy on day 10, 15, 20, 25 and 28 following larviposition. After irradiation, pupae were kept together with untreated pupae in the insectary to complete development. Daily examinations were made on fly emergence and non emerged pupae were dissected and their content examined (Bursell, 1959). Male and female fertility was assessed as described in chapter 2. Sperm motility was checked by visual observation under the compound microscope of ruptured spermathecae. Performance parameters and survival of females were checked for a period of 40-45 days following emergence. Adult male survival was assessed by recording daily mortality until all flies had died.

RESULTS

Eclosion of irradiated and untreated pupae

Table 1 presents the emergence data of the various batches of treated and untreated pupae. Under the prevailing rearing conditions, female and male pupal period lasted on average 31.8 and 33.9 days respectively. The time required for female and male flies to complete their full development was not altered by the treatment. Treating pupae on days 28, 25 post larviposition (PL) with doses up to 120 Gy and on day 20 and 15 PL with doses up to 80 Gy and 40 Gy respectively, resulted in emergence rates comparable with emergence of untreated pupae (> 81%). A treatment of 100 and 120 Gy on day 20 PL reduced the emergence rate to 64% and 54.5% respectively. Males were more susceptible to the radiation treatment with only 42.1% and 16.6% emerging for the two doses respectively. However, there were no indications that the treatments killed the males at the moment of irradiation as they continued their development until shortly before emergence. Twelve males in the 120 Gy and 8 males in the 100 Gy group managed to rupture the puparium, but failed to force their way out and died. A 100 - 120 Gy treatment of 15 day old pupae was fatal for all males, although dissections of the non emerged pupae revealed a complete development. Female emergence was only affected when rather young pupae (15 PL) were treated with high doses of 100 and 120 Gy. Except for the 10 Gy treatment, gamma radiation killed most of the 10 day old puparia.

Fecundity and survival of male flies emerged from treated pupae

Reproduction of colony females mated with males treated as pupae is presented in Table 2. Male fertility is expressed as the number of pupae produced per mature female day (the number of mated females being able to produce viable offspring). Insemination capacity and sperm motility was not affected in males treated on day 28 PL for all treatment doses and on day 25 PL with doses up to 100 Gy. Although males treated with high doses of 120 Gy on day 25 PL and with low doses of 10 Gy on day 10 PL displayed a normal mating behaviour with

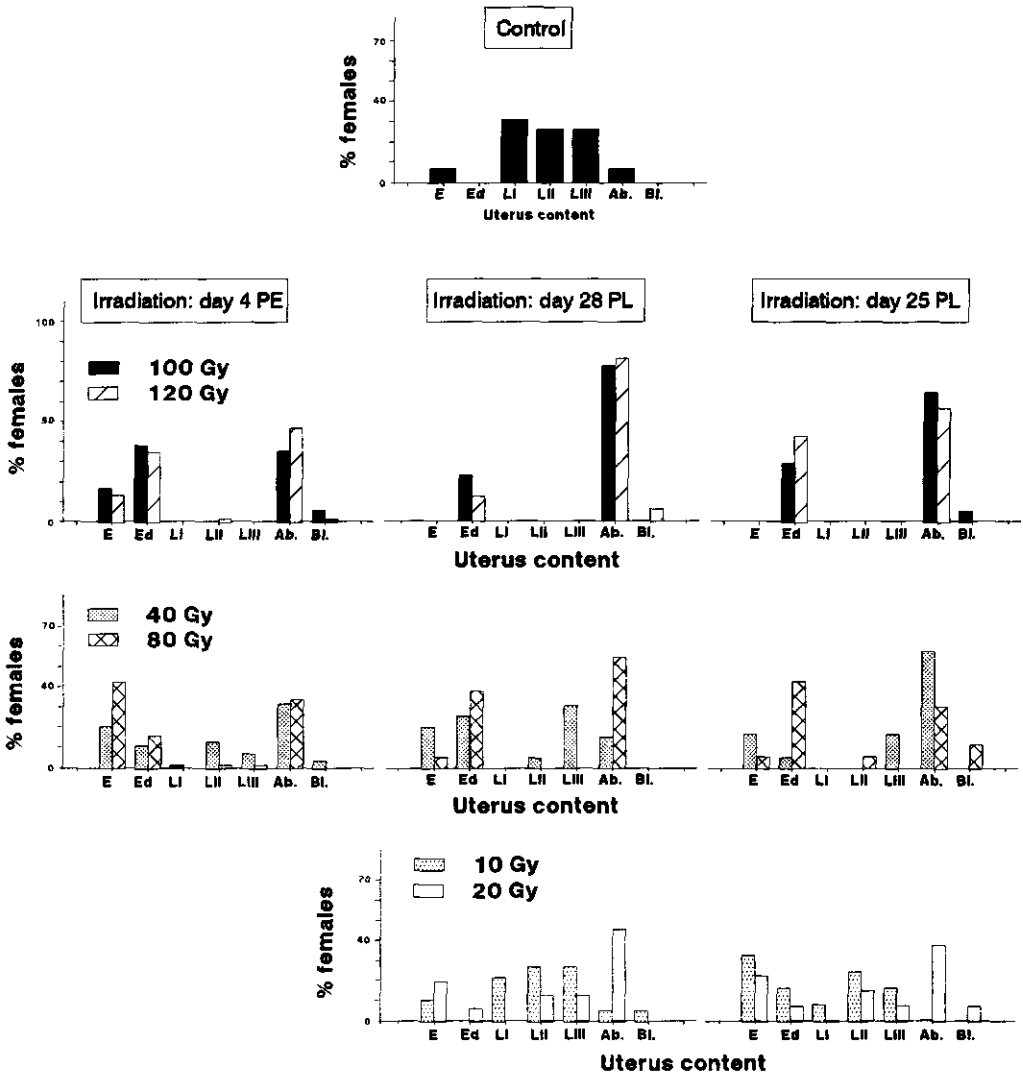

Fig. 1. Reproductive status of female *G. tachinoides* mated with males irradiated (doses between 10 and 20 Gy) as pupae on day 25 and 28 post larviposition (PL) and as adults on day 4 following emergence (PE) as compared with females mated with untreated colony males. (Uterus content: E = recently ovulated egg, Ed = degenerating egg, LI, LII, LIII = first, second and third instar larva, Ab. & BI. = uterus empty due to abortion of egg/immature larva or blockage of ovarioles)

Table 3. Average longevity of male *G.tachinoides*, irradiated during various phases of the pupal development

Irradiation day [1]	Dose (Gy)	No. males	Average life span ± SD (days)	Sign. [2]	Survival (%) on indicated days following emergence				
					20	30	40	50	60
Control	0	132	47.9 ± 24.3		80.3	68.9	60.6	55.3	42.4
28	10	22	48.3 ± 22.3	NS	77.3	77.3	59.1	45.5	40.9
	20	23	42.9 ± 21.0	NS	69.9	69.9	47.8	39.1	26.1
	40	18	36.7 ± 17.1	*	55.6	55.6	55.6	16.7	16.7
	80	20	31.6 ± 18.1	***	40.9	31.8	22.7	13.6	13.6
	100	22	24.2 ± 8.7	***	31.8	22.7	9.1	0.0	0.0
	120	22	26.2 ± 9.5	***	40.9	36.4	4.5	0.0	0.0
25	10	24	51.7 ± 27.3	NS	79.2	66.7	66.7	62.5	41.7
	20	24	51.4 ± 25.3	NS	87.5	66.7	66.7	62.5	37.5
	40	26	28.9 ± 17.0	***	69.2	26.9	19.2	15.4	3.8
	80	31	23.7 ± 15.9	***	42.4	15.2	12.1	9.1	3.0
	100	33	19.6 ± 6.3	***	48.5	6.1	3.0	0.0	0.0
	120	40	15.8 ± 3.6	***	17.5	0.0	0.0	0.0	0.0
20	10	34	47.9 ± 22.4	NS	73.5	70.6	58.8	50.0	32.4
	20	41	28.7 ± 24.3	***	41.5	31.7	31.7	26.8	14.6
	40	36	9.1 ± 0.3	***	0.0	0.0	0.0	0.0	0.0
15	10	32	29.6 ± 21.5	***	50.0	40.6	21.9	18.8	15.6
	20	31	5.5 ± 2.7	***	0.0	0.0	0.0	0.0	0.0
10	10	41	17.3 ± 17.0	***	24.4	19.5	9.8	7.3	7.3

1. Days post larviposition
2. Significant difference ($p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), t test) from control
   NS = Not significant

all female mates showing mating scars, only 70% and 61.5% of the females were found inseminated. All females mated with males treated with low doses of 10 - 20 Gy during their mid-pupal phase i.e. day 15 - 20 PL, were found with motile sperm in the spermathecae.

A higher level of sterility was obtained after treatment of 25 - 28 day old male pupae when compared with sterility levels of males treated with similar doses as adults (Vreysen *et al.*, submitted) i.e. a dose of 100 Gy and 80 Gy administered to males on day 28 and 25 post larviposition respectively induced complete sterility in the female mates (adults require a 140 Gy treatment for complete sterility). A residual fertility of more than 19% relative to the control was obtained for males treated with 10 Gy on day 15 and day 20 PL. Treating 20 day old male pupae with 20 Gy however induced 95% sterility in their female mates.

No significant differences were observed between the average weight of the pupae produced by females mated with males irradiated in the late pupal phase (25 - 28 PL) as compared with pupae produced by the control females (except for pupae fathered by males treated with 10 Gy on day 25 PL). All pupae fathered by males irradiated with doses of 10 and 20 Gy on day 20 or earlier weighed significantly less than pupae produced by control females.

Dissection (on day 45) of the females, mated with males emerged from treated 25 - 28 day old pupae, revealed a pregnancy picture similar to the one found in females mated with males treated as adults (Chapter 2) (Fig. 1). None of the inseminated females, mated with males irradiated with 80 - 120 Gy on day 28 PL and with doses of 100 - 120 Gy on day 25 PL were found with a viable larva *in utero*. The uterus of these females (i) contained a recently ovulated egg or one or more eggs in embryonic arrest, or (ii) was found empty due to expulsion of the dead embryos or due to blockage of the ovarioles preventing further ovulations. The residual fertility of males treated with low doses of 10 - 40 Gy was reflected in pregnancy stages up to the third instar larva in the dissected female mates.

Survival of the males emerged from irradiated pupae is presented in Table 3. Only males treated with 10 - 20 Gy as 25 - 28 day old pupae and with 10 Gy as 20 day old pupae had a survival comparable with untreated males (average longevity > 42 days). Higher treatment doses (40 Gy or more) administered during the later development stages and a dose of 20 Gy or more given to 20 day old pupae reduced significantly the average longevity of the males. The same observation was made with a treatment of 10 Gy given to 10 - 15 day old pupae.

The optimal sterilising dose in relation to pupal age was assessed by

plotting male survival data against their fertility (Fig. 2). An average life span of 21 days i.e. 44% of the life span of untreated control males, was considered to be the minimum acceptable in conjunction with a residual fertility of 5% relative to the untreated control. Only males treated as 28 day old pupae with 100 - 120 Gy, as 25 day old pupae treated with 80 Gy and as 20 day old pupae with 20 Gy showed a correlation between fertility/survival fitting the criteria. Males emerged from all other treatment and pupal age groups contained either sperm bearing a too high residual fertility or showed inferior/sub-optimal survival rates due to radiation induced somatic damage. These data indicate that irradiation of *G. tachinoides* pupae in air in the last 10 days of pupal development does not affect the longevity, the mating and insemination capacity of the male flies.

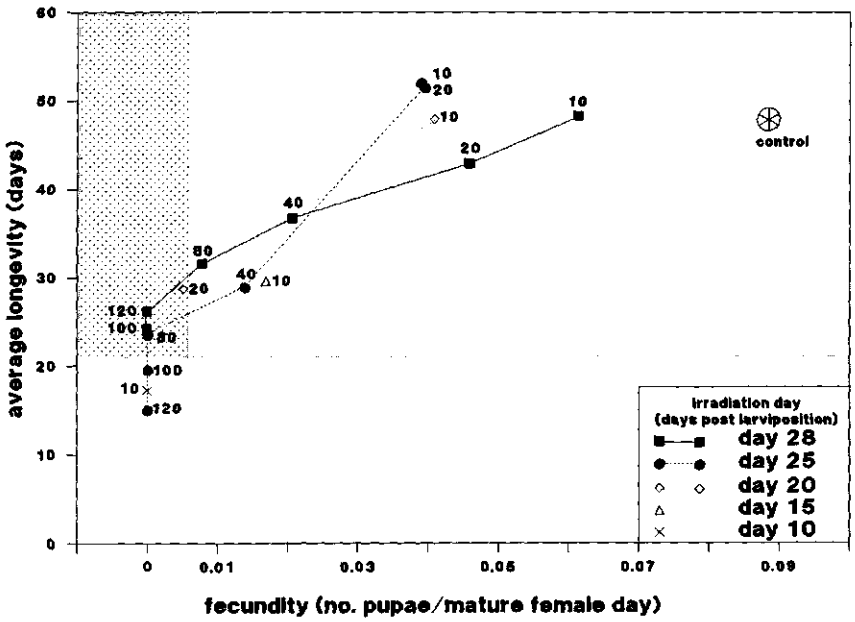

Fig. 2. Fertility and average longevity of male *G. tachinoides* irradiated as pupae on day 10, 15, 20, 25 and 28 following larviposition. Numbers on graph indicate the irradiation dose in Gy. Shaded area indicate males with an average longevity above 21 days and a residual fertility of ≤ 5% relative to untreated control males.

Table 4. Fertility of female *G.tachinoides* irradiated with doses of 10 - 120 Gy during various phases of their pupal development and mated with untreated colony males

Irradiation day / dose [1] (Gy)	Initial females no.	Female survival day 40 [2]	Mating status MS + / SP + % [3]	Fecundity [4]	Production relative to control	No. of eggs recovered on day 20	30	40	Emergence/ females %
Control	91	95.9	100 / 95.0	0.093	100	16	10	8	80.9 / 56.5
28 / 10	23	95.1	100 / 83.3	0.023	24.6	21	27	10	80.0 / 50.0
20	19	92.1	100 / 95.4	0.006	6.4	17	17	8	1/2 / 1/1
40	22	99.6	92.0 / 92.0	0.002	2.2	18	17	2	---
80	27	88.5	100 / 95.4	0.000	0.0	18	11	1	---
100	23	100	100 / 84.2	0.000	0.0	18	7	0	---
120	21	91.6	100 / 95.6	0.000	0.0	13	5	1	---
25 / 10	25	75.6	95.0 / 95.0	0.014	14.8	23	22	6	2/5 / 2/2
20	23	99.3	100 / 95.2	0.011	11.8	20	9	11	2/5 / 1/2
40	23	100	100 / 100	0.000	0.0	19	7	0	---
80	20	100	100 / 95.6	0.000	0.0	10	1	0	---
100	23	95.4	100 / 86.9	0.000	0.0	8	0	0	---
120	20	100	100 / 90.9	0.000	0.0	2	0	0	---
20 / 10	20	100	100 / 92.3	0.010	10.4	11	18	5	3/4 / 1/2
20	20	100	100 / 92.3	0.014	15.6	0	3	7	3/5 / 2/3
40	23	100	100 / 91.6	0.000	0.0	0	0	0	---
80	17	99.6	100 / 82.6	0.000	0.0	0	0	0	---
100	19	96.3	88.2 / 88.2	0.000	0.0	0	0	0	---
120	17	100	100 / 100	0.000	0.0	0	0	0	---
15 / 10	24	100	100 / 100	0.035	37.5	15	12	2	93.8 / 33.3
10 / 10	32	100	100 / 100	0.000	0.0	0	7	5	---

1. Days post larviposition
2. Survival relative to mature female days
3. MS +: Mating Scars present
   SP +: Spermathecae impregnated with motile sperm
4. No. pupae per mature female day

Fecundity and survival of female flies emerged from treated pupae

Survival and mating response of females was not affected when treated as 20, 25 and 28-day old pupae with radiation doses up to 120 Gy (Table 4). All females emerging from pupae treated on day 15 PL or at younger stages with doses exceeding 10 Gy, died during the first days following emergence. In all the experimental groups, motile sperm was found in more than 83% of the females. No offspring was produced by females treated with doses exceeding 40 Gy as 28 day old pupae and with doses exceeding 20 Gy as 20 and 25 day old pupae. Pupae produced by females irradiated with 10 - 20 Gy always weighed significantly less as compared with pupae produced by untreated control females (except pupae produced by the females irradiated with 10 - 20 Gy on day 20 PL). Treating females during the mid-pupal phase (day 15 PL) with low dose radiation of 10 Gy caused little somatic damage and resulted in viable females with a normal mating response and mating behaviour. However, a total of 0.035 pupae/mature female day were produced i.e. a residual fecundity of 37.5% as compared with control females.

The number of expelled eggs decreased with increasing radiation doses i.e. females treated with 10 - 20 Gy as 28-day old pupae and with 10 Gy as 25-day old pupae expelled more than 2 eggs during the 40-day experimental period whereas the number of expelled eggs was reduced to 1.1 and 0.1 eggs/mature female for females treated with 120 Gy on day 28 and 25 PL respectively. No eggs were aborted by females treated as 20 day old pupae with doses of 40 Gy and more. Treating 10 and 15 day old pupae with a dose of 10 Gy resulted in 2.4 and 1.5 eggs expelled per mature female respectively.

Dissection of the females after 40 days revealed that 95% of the females irradiated as 20 - 28 day old pupae with doses of 40 Gy or more displayed completely or partially atrophied ovaries. The latter were characterised by inactivated left or right ovarioles, but with visible differentiation between oocyte and trophocytes of the follicle next in ovulation sequence A.C.B.D. with however the oocyte maturation process interrupted. Between 5 and 16% of females treated with 20 Gy revealed a normal pattern in configuration of the ovarioles and uterus content. More than 70% of females treated with 10 Gy irrespective of the treatment age, revealed a normal reproductive status.

DISCUSSION

Our data on male and female eclosion of *Glossina tachinoides* pupae treated with doses between 10 and 120 Gy indicate that at younger stages *G. tachinoides* males are more susceptible to ionising radiation than females. Whereas treatments up to 120 Gy did not adversely affect female emergence in pupae as young as 20 days, doses of above 80 Gy reduced significantly viability of 20 day old male pupae. The data corroborate results obtained with irradiated *G. m. morsitans* (Dean & Wortham, 1969; Langley *et al.*, 1974) and *G. palpalis palpalis* pupae (Van der Vloedt *et al., 1976*).

The biological quality of gamma sterilised male flies is primarily determined by their mating vigour, mating behaviour and their capacity to inseminate wild female flies with competitive motile sperm. A female mated with a sterile male without receival of sperm for storage in the spermathecae can subsequently become inseminated by a fertile male due to the female's potential multiple mating behaviour (Jordan, 1958; Van der Vloedt *et al.*, 1978; Vreysen & Van der Vloedt, 1992). Therefore, in sterilising male tsetse flies as pupae it is a prerequisite that the gamma radiation treatment does not interfere with the production of mature motile sperm. Our observations on the formation of the male reproductive organs and the process of spermatogenesis in *G. tachinoides* pupae under the described rearing conditions (unpublished data), largely corroborate the data of Itard (1970) i.e. meiosis occurs between day 6 and 9 with the formation by day 11 of young spermatids characterised by a short flagellum, round head and big nucleus. The spermiogenesis process continues between day 12 and 18 with mature spermatozoa appearing on day 20 - 21. At the time of eclosion, spermatogenesis has already reached completion and male tsetse flies emerge with their entire supply of mature sperm.

It is well known that the stage of cell development at the time of irradiation is the major factor that influences radiosensitivity. Any radiation treatment administered during the first third of the pupal development coincides with periods of intense cell division leading to radiation induced spermatogonial death. In addition, due to excessive somatic damage, high mortality in the puparium is observed. During the mid-pupal phase (day 15 - 20) i.e. the period of spermiogenesis, low dose treatments of 10 Gy did not adversely affect the process of sperm maturation. Moreover, these males were capable of transferring viable sperm but with a residual fertility between 19 and 46%. Increasing the dose rate to 20 Gy caused a high incidence of dominant lethal mutations (5% residual fertility) in the sperm of 20 day old male pupae but dose

rates exceeding 40 Gy resulted in a high pupal death or adult mortality during the first 7 days after emergence. Finally, in 25 - 28 day old pupae, spermiogenesis has been completed and even treatments up to 120 Gy did not hamper sperm function or caused sperm inactivation. In all female mates, aberrations in the uterine content and the ovarian configuration were in direct proportion to the age at which the male received treatment and to the treatment dose.

Irradiating 2-4 day old adult male *G. tachinoides* (chapter 2) required higher radiation doses to obtain the same level of sterility as compared with males irradiated as 28 day old pupae. This in spite of the fact that mature sperm is formed by day 22 and no obvious changes occur afterwards. The reasons for these differences in radiosensitivity remain speculative and possibly, non genetic factors are involved.

Curtis & Langley (1982) have stated "the importance of gamma sterilised males having an as long and active life as possible because the chances of encountering a receptive female are very limited in the field". As observed with other tsetse species, survival of *G. tachinoides* males treated as pupae decreased with increasing doses and with decreasing pupal age at the time of treatment. For the same amount of induced sterility however, survival of males, irradiated as pupae is lower as compared with males irradiated as adults (Chapter 2). Whereas Langley *et al.* (1974) determined that 27 day old *G. m. morsitans* pupae are the youngest stages which can be treated in air to obtain sterile and competitive males, our data show that 20 day old *G tachinoides* male pupae can be treated with 20 Gy in air resulting in a residual fertility of 5% and with an average longevity above 3 weeks. However, a radiation treatment in air of *G. tachinoides* pupae in the mid pupal phase (15 day old pupae) seems not feasible as it leads to a high degree of somatic damage or too low incidence of dominant lethal mutations. Consequently, long distant transportation of *G. tachinoides* pupae irradiated in air, seems limited in view of the short period between irradiation treatment and onset of female eclosion. The option of treating pupae at earlier pupal stages in a nitrogen atmosphere will be explored in a next paper.

Previous research has already shown that low dose irradiation administered to 2-4 day old adult female *G. p. palpalis* and *G. austeni* or to 33 day old *G. austeni* pupae (Van der Vloedt & Barnor, 1984; Vreysen & Van der Vloedt, 1992), resulted in a complete loss of female fertility. Our data on irradiation of female *G. tachinoides* pupae resemble those results since a treatment of 20 - 28 day old female pupae with 40 Gy induced complete sterility in the adults. When pupae were irradiated at younger stages only viable females were obtained

when a 10 Gy treatment was given to 15 day old pupae. This low dose treatment however, resulted in a residual fertility of 37.5%. The high rate of induced lethal mutations in the oocyte and/or inhibition of ovarian growth together with extreme good survival rates of females irradiated as 20 - 28 day old pupae with 40 to 80 Gy indicate the potential of using these flies as tracer insects in release recapture studies to expose low density fly populations (Van der Vloedt & Barnor, 1984; Vreysen & Van der Vloedt, 1992).

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Chapter 4

RADIATION STERILISATION OF *Glossina tachinoides* Westwood PUPAE: II. THE EFFECT OF DOSE FRACTIONATION AND NITROGEN DURING IRRADIATION IN THE MID PUPAL PHASE

Abstract

A study was carried out to analyse the effect of nitrogen during radiation and dose fractionation on *Glossina tachinoides* pupae during the mid pupal phase (day 15 - 20 following larviposition (PL)). The radiation protective effect of nitrogen during treatments of 10 - 80 Gy of 15 - 20 day old pupae was demonstrated by an increased total eclosion rate (for 15 day old pupae), higher residual male fertility levels (34.6% and 44.6% for 15 and 20 day old pupae treated in nitrogen respectively versus 19.1% and 5.9% for treatments in air) and distinctive longer life spans. The proportion of reproductive abnormalities observed in their female mates increased with increased radiation dose, when treated at younger pupal stages and following treatment in air. After treatment of 15 day old pupae with 10 Gy in nitrogen, female fertility was 0.068 pupae per mature female day as compared to 0.035 pupae/m.f.d. in air. No such increase was observed when treated as 20 day old pupae. A dose of 60 - 80 Gy in nitrogen administered to 20 day old female pupae was required to obtain 95% sterility.

Splitting the radiation dose in nitrogen atmosphere in 2 fractions 1, 2 and 5 days apart (first dose of 10 Gy given on day 15 PL) did not influence the total eclosion rate, mating response and insemination capacity of the male flies. Sterility of males treated in fractions separated by 1 and 2 days was similar to the ones given a continuous dose on day 15 PL but the level of induced lethal mutations decreased with fractions separated by 5 days. Survival of the males treated in fractionated doses was similar as compared to males treated with one continuous dose on day 20 PL but better when compared to males treated with one continuous dose on day 15 PL. Female fecundity was reduced by splitting the radiation dose in fractions 1 and 2 days apart. Complete sterility was induced in female pupae when fractions were separated by 5 days, irrespective of the radiation dose. Irradiation of *G. tachinoides* pupae in the mid pupal phase in nitrogen with doses split in 2 fractions separated by 1 or 2 days (total dose of 40 Gy) or 5 days (total dose of 60 - 80 Gy) resulted in high quality (average longevity > 20 days) sterile (residual fertility < 5%) male flies.

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INTRODUCTION

Both laboratory studies and operational field programmes using the Sterile Insect Technique (SIT), have indicated the feasibility of deploying male tsetse flies (Glossinidae) of the *morsitans* and *palpalis* group, sterilised by ionising radiation in the pupal stage (Curtis & Langley, 1972; Van der Vloedt *et al.*, 1976, Williamson *et al.*, 1983d). A prerequisite for the successful application of the sterile insect technique is the release of highly competitive sterile insects with a behaviour similar to the one of the wild insects. Previous research on the effect of gamma irradiation on *Glossina tachinoides* pupae has shown that radiation treatment in air is limited to the last third of the pupal development (chapter 3). Attempts to obtain viable high quality sterile males by treating pupae during the mid pupal phase with doses of 10 - 120 Gy failed due to (1) an increased rate of early pupal death due to radiation induced somatic damage, (2) reduced survival of adult males in the first week after emergence, (3) inferior insemination capacity of the males or (4) sub-optimal sterility levels with low dose radiation treatment. These results made us wonder whether the conditions under which the flies were irradiated led to the observed effects. Curtis & Langley (1972) and Langley *et al.* (1974) have demonstrated that irradiation under low oxygen tension e.g. in a nitrogen atmosphere, reduced not only the amount of induced dominant lethals in *G. m. morsitans* males, treated in the late pupal phase, but also the level of somatic damage. The effects of dose fractionation on *G. m. morsitans* pupae of unknown age have in addition been described (Dean & Wortham, 1969). This has prompted us to examine the effect of nitrogen and dose fractionation on the radiation susceptibility of female and male *G. tachinoides* pupae during their mid pupal phase of development. Reproduction and survival of both sexes was studied in relation to treatment atmosphere, treatment age and interval between fractions.

MATERIAL AND METHODS

Pupae and fly material

All pupae used for the experiments were derived from the *Glossina tachinoides* colony, originating from the Central African Republic, and maintained on a membrane feeding system at the Entomology Unit of the IAEA 's laboratory in Seibersdorf, Austria. Pupae and adult flies were kept under normal colony conditions i.e. 23° ± 1°C and 75% ± 5%

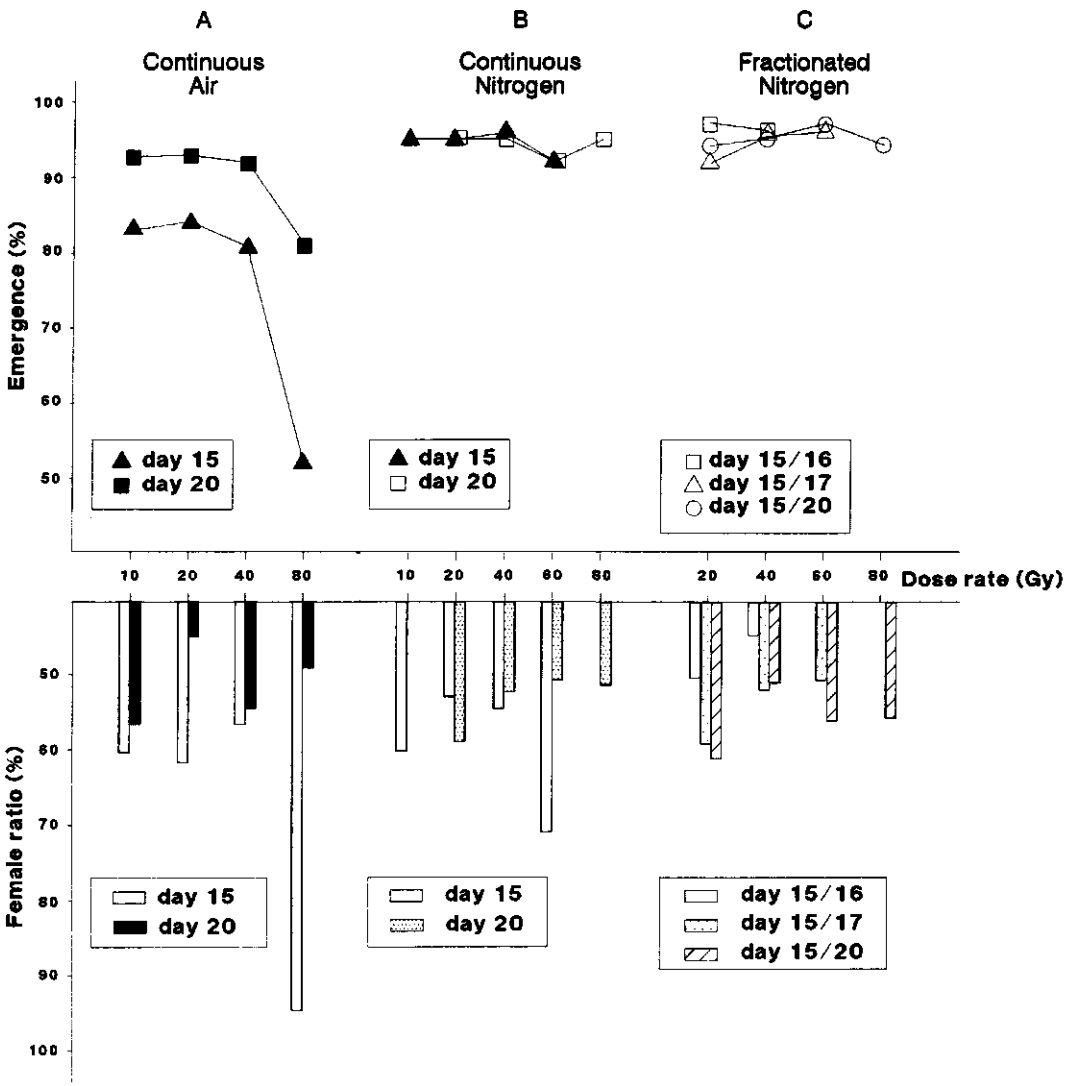

Fig. 1 Emergence rate and female ratio of *G. tachinoides* pupae irradiated during the mid pupal phase in air (A) and nitrogen (B) on day 15 and 20 following larviposition and in doses split into two fractions (C). (1 st dose of 10 Gy given on day 15 PL, 2 nd dose 1,2 or 5 days interval)

Relative Humidity. All flies were fed daily (except on Sundays) on equal proportions of frozen and thawed bovine and porcine blood (Wetzel & Luger, 1978).

Radiation procedures and experimental design

Batches of 100 pupae, collected on the same day and kept in petri-dishes (diameter 5.5 cm, height 1.5 cm), were exposed in a ^{60}Co source (dose rate of 6 Gy/min.) to irradiation doses ranging between 10 and 80 Gy in air and nitrogen on days 15 and 20 following larviposition. When nitrogen was used, the batches of pupae were transferred from the plastic holding petri-dishes to the bottom of a glass container as described by Economopoulos (1977). For 15 - 20 minutes, nitrogen flowed through the inlet leading to the bottom of the device, allowing the oxygen to escape through the outlet. Pupae were irradiated in the device and after irradiation transferred to plastic petri-dishes for completion of development. Maximum exposure of the pupae to the nitrogen atmosphere was 30 minutes.

All dose fractionation experiments were carried out under nitrogen atmosphere. Doses were split into 2 fractions separated by 1, 2 or 5 days, whereby the first dose of 10 Gy was administered on day 15 post larviposition (PL). Dose rates ranging between 10 and 70 Gy were applied for the second fraction.

Together with each experimental group, a batch of untreated control pupae was kept under standard colony conditions. Emerging flies were collected daily and transferred to standard fly holding cages. Mating procedures and experimental designs to assess male and female reproductive parameters were used as described in a previous paper (Chapter 2). Male survival was assessed by recording daily mortality but the observations ended after 80 days.

RESULTS

Effect of nitrogen

Eclosion of male and female pupae irradiated on day 15 and 20 PL in air and nitrogen atmosphere with doses ranging from 10 to 80 Gy are presented in Fig. 1. The percentage adult eclosion of pupae irradiated on day 15 in air was significantly reduced as compared to pupae irradiated in nitrogen and control pupae (chi square, $p < 0.01$). No such atmosphere related differences in eclosion rate were found with 20 day old pupae

Table 1. Fertility of male *G. tachinoides* irradiated in air, nitrogen, in single and in fractionated doses and mated with untreated colony females

Irradiation 1 day/atm./dose [1]	Irradiation 2 day/atm./dose	Initial females no.	Female survival day 45 % [2]	Mating status MS + / SP + % [3]	Mean puparial weight (mg) ± SD	Fecundity [4]	Production relative to control	No. aborted eggs	Emergence/ females %
Control		40	93.8	95.0 / 87.2	18.7 ± 2.8	0.091	100	21	90.0 / 44.4
15 / A / 10	-- / -- / --	20	100	100 / 100	15.2 ± 3.2	0.017	19.1	67	88.9 / 37.5
20 / A / 20	-- / -- / --	31	98.2	100 / 100	12.6 ± 1.7	0.005	5.9	91	3 / 4 / 1 / 3
15 / N / 10	-- / -- / --	32	91.9	100 / 100	14.5 ± 2.9	0.032	34.6	53	88.5 / 91.7
20	-- / -- / --	30	88.2	100 / 100	16.1 ± 2.6	0.024	26.6	54	77.8 / 50.0
40	-- / -- / --	20	90.4	100 / 100	-----	0.000	2.2	50	1 / 1 / 1 / 1
20 / N / 20	-- / -- / --	35	97.5	95.0 / 100	15.8 ± 1.6	0.041	44.6	49	84.0 / 19.0
40	-- / -- / --	40	87.5	100 / 100	16.1 ± 2.1	0.025	27.9	62	87.5 / 31.3
60	-- / -- / --	40	97.5	95.0 / 90.0	15.2 ± 1.3	0.009	9.3	81	5 / 6 / 1 / 5
80	-- / -- / --	40	91.5	95.0 / 90.0	16.2 ± 2.2	0.009	10.0	85	5 / 6 / 1 / 5
15 / N / 10	16 / N / 10	40	79.0	100 / 100	15.6 ± 2.5	0.022	23.6	70	68.4 / 30.8
	30	30	80.0	96.3 / 92.6	17.3 ± 0.6	0.005	5.4	76	2 / 3 / 1 / 2
15 / N / 10	17 / N / 10	30	56.8	96.0 / 84.0	15.6 ± 2.4	0.025	27.1	41	8 / 9 / 2 / 8
	30	30	82.0	96.2 / 96.2	15.2 ± 2.2	0.006	6.7	64	4 / 4 / 1 / 4
15 / N / 10	20 / N / 10	35	95.4	100 / 100	16.5 ± 2.5	0.032	34.6	62	84.2 / 37.5
	30	40	100	100 / 100	15.1 ± 0.6	0.013	13.7	87	7 / 9 / 3 / 7
	50	35	97.6	100 / 100	11.6 ± 2.4	0.003	3.6	90	1 / 2 / 1 / 1
	70	35	94.8	100 / 95.0	---	0.002	1.9	91	1 / 1 / 0 / 1

1. Day: Days post larviposition, Atm.: Irradiation atmosphere (A = Air, N = Nitrogen), Dose: Gy
2. Survival relative to mature female days
3. MS +: Mating Scars present
   SP +: Spermathecae impregnated with motile sperm
4. No. pupae per mature female day

(chi square, $p > 0.05$) except when the irradiation dose was increased to 80 Gy (chi square = 27.70, $p < 0.01$). Whereas the majority of the male flies was killed when treated in air as 15 day old pupae with a dose of 80 Gy (chi square = 38.88, $p < 0.01$) no such adverse effect was observed when an irradiation treatment of 60 Gy was administered in nitrogen (chi square = 0.015, $p > 0.05$).

Fecundity of untreated females mated with males irradiated as 15 - 20 day old pupae is presented in Table 1. Irradiation in nitrogen resulted in a substantial increase in male residual fertility as compared to irradiation in air. In addition, this increase in male fertility was related to the pupal age when treatment was given i.e. irradiation in nitrogen of 15 day old pupae resulted in a twofold increase of male fertility versus an eightfold fertility increase for males irradiated as 20 day old pupae. Fertility was decreased for the same radiation dose given at younger stages. Insemination capacity of the irradiated males was not affected, irrespective of the age of the male pupae during treatment, dose rate or irradiation atmosphere. This was evidenced by 90 to 100% of the females, mated with treated males, displaying spermathecae impregnated with motile sperm versus 85.7% of the control females. The average weight of all experimental pupae was significantly reduced as compared with pupae fathered by control males (Student's *t* - test, $p < 0.01$). Viability of the offspring was comparable with the controls (chi square, $p > 0.05$) but whereas irradiation in air gave almost equal proportions of males and females, offspring of the pupae irradiated in nitrogen on day 20 were biased in favour of males (significant only for 20 Gy treatment group, chi square = 6.88, $p < 0.01$). Remarkably, no such bias was observed in offspring fathered by males irradiated in nitrogen on day 15 PL. On the contrary, offspring fathered by males irradiated with 10 Gy were mostly females (91.7%)(chi square = 14.37, $p < 0.01$). Analysis of the reproductive status of the female mates revealed data confirming the fertility results i.e. the percent observed reproductive abnormalities due to radiation induced sterility (and consequently the number of expelled dead embryos) was correlated with (1) pupal age when treatment was given, (2) irradiation atmosphere and (3) radiation dose (Fig.2). About 70% of females mated with males irradiated in air as 15 and 20 day old pupae with doses of 10 and 20 Gy respectively showed an uterus empty due to expulsion of the degenerating egg or a degenerating egg *in utero*, whereas in a nitrogen atmosphere, a dose of 40 Gy was required to obtain the same amount of aberrations in the ovarian configuration and the uterus content.

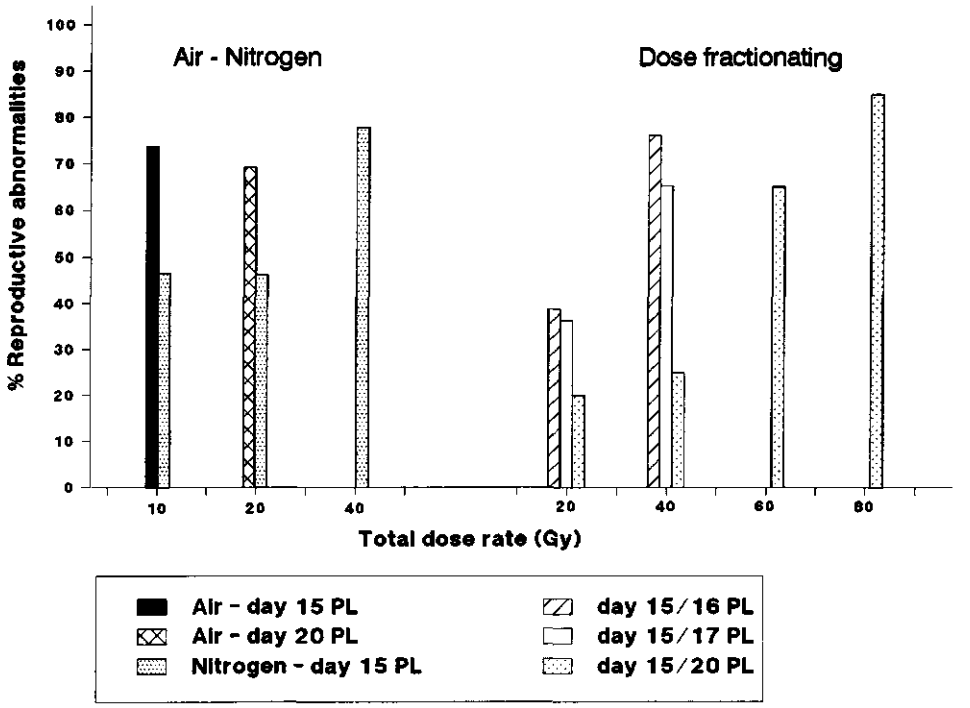

Fig. 2 Frequency distribution of reproductive abnormalities in female *G. tachinoides* mated with males irradiated as pupae in air and nitrogen atmosphere in doses split in 2 fractions 1, 2 and 5 days apart.

Survival curves of untreated males and males treated as 15 - 20 day old pupae in air and nitrogen are presented in Fig. 3. Survival of males irradiated in nitrogen was dramatically increased as compared with males irradiated in air. Whereas after 20 days already 50% and 58.5% of the males, irradiated with 10 - 20 Gy in air as 15 - 20 day old pupae respectively had died, mortality was only 21.1% and 10.8% when the same treatment was given in nitrogen. Survival curves of control males resembled those of males irradiated in nitrogen on day 20 PL with doses up to 60 Gy (> 50% of the males surviving day 70). When the dose was increased to 80 Gy, 50% of the males were still alive on day 58 following emergence. Moreover, males treated on day 20 PL with a dose

of 20 Gy in nitrogen survived better than control males (LT_{50} of 81 days versus 75 for control males).

Fecundity of females, irradiated as 15 day old pupae, increased from 0.035 pupae per mature female day (37.5% residual fertility) when a 10 Gy treatment was given in air to 0.068 pupae/mature female day (74.8% residual fertility) when the same irradiation dose was given in nitrogen (Table 2). No such nitrogen related increase in fertility occurred when treated on day 20 PL (residual fertility decreased from 15.6% to 8.3% for females irradiated with 20 Gy in air and nitrogen respectively). A high degree of sterility (> 95%) was only observed in females treated in nitrogen with 60 and 80 Gy as 20 day old pupae. Mating receptivity of females of all experimental groups was normal (> 95% mating scars) except for the females irradiated in nitrogen with 40 Gy as 15 day old pupae. The number of expelled eggs per mature female decreased from 1.5 for females treated in air with 10 Gy as 15 day old pupae to 0.7 for treatments in nitrogen but increased from 0.6 to 0.9 for a treatment of 20 Gy given to 20 day old pupae. The number of expelled immature larvae however, increased from 0.1/mature female for females treated on day 15 PL in air to 0.4/mature female for females treated in nitrogen.

Effect of dose fractionating

Splitting the radiation dose in 2 fractions separated by 1, 2 or 5 days had no significant effect on the eclosion rate of both male and female flies (Fig. 1). More than 92% of all the experimental pupae emerged which was comparable with the eclosion rate of the untreated pupae (chi square, $p > 0.05$). Female reproduction rates were similar when doses were split in fractions separated with 1 or 2 days as compared to reproduction of females mated with males who had received the same dose rate in a single dose on day 15 PL (Table 1). Increasing the time interval between the first and second dose to 5 days resulted in a higher residual male fertility as compared with fertility of males given a continuous dose on day 15 PL, but slightly less as compared to males irradiated in a single dose on day 20 PL. Dissections revealed that males displayed a normal mating vigour (> 93% of females with mating scars) and insemination capacity with more than 90% of their female mates being inseminated (with the exception of males irradiated in split doses (20 Gy) 2 days apart). Average weight of produced pupae was comparable with weight of pupae fathered by males receiving a continuous dose ($p > 0.05$). Although the sex ratio of offspring was in

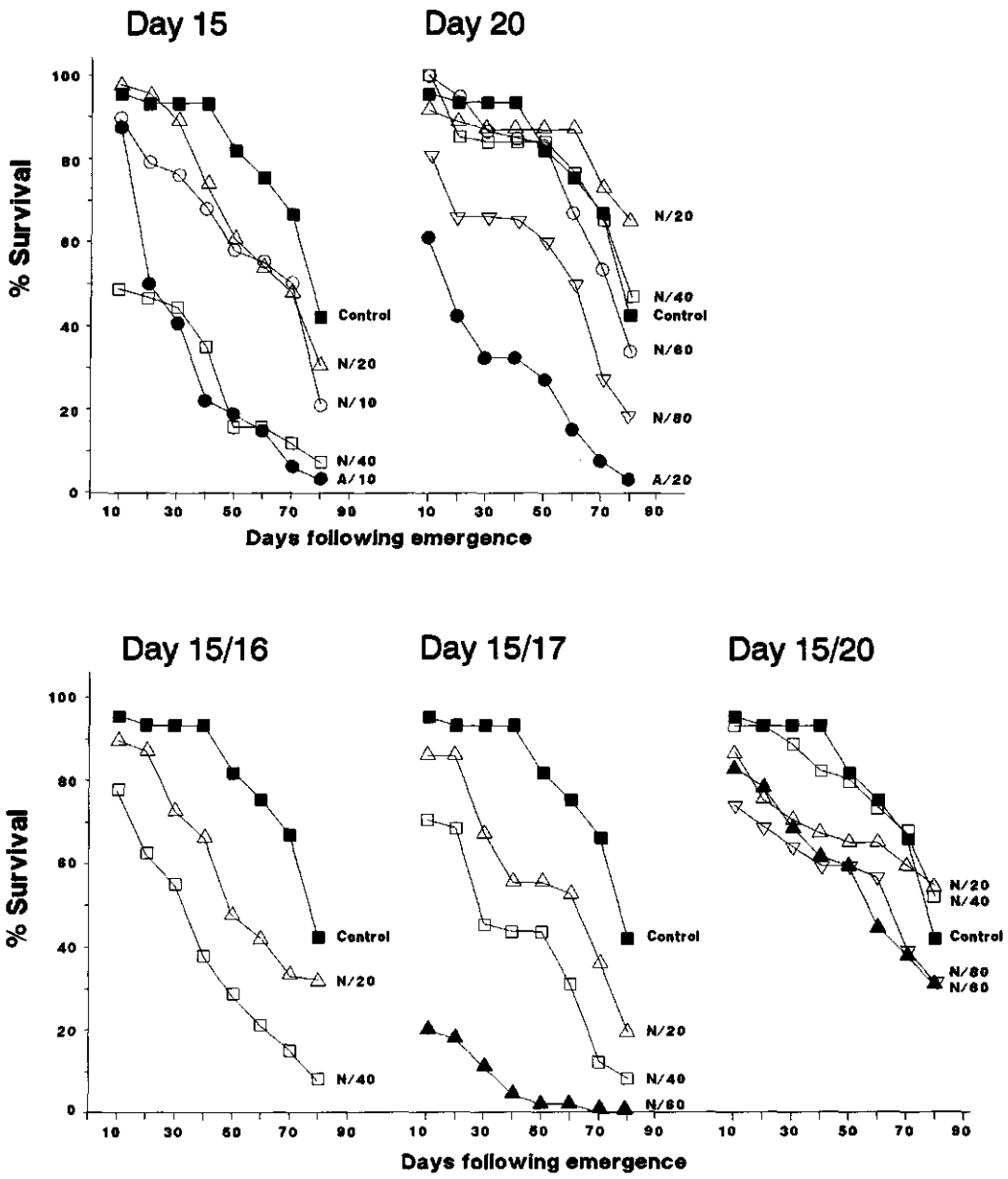

Fig. 3 Survival curves of male *G. tachinoides* irradiated with continuous dose in air (A) and nitrogen (N) atmosphere on day 15 and 20 following larviposition (top graphs) and with doses split into two fractions (bottom graphs). (First dose of 10 Gy was administered on day 15 post larviposition, second was given 1, 2 and 5 days apart). Figures on graph indicate total dose received (in Gy).

Table 2. Fertility of *G. tachinoides* females, irradiated in air, nitrogen, in single and in fractioned doses and mated with untreated colony males

Irradiation 1 day/atm./dose [1]	Irradiation 2 day/atm./dose	Initial females no.	Female survival day 40 % [2]	Mating status MS + / SP + % [3]	Mean puparial weight (mg) ± SD	Fecundity [4]	Production relative to control	No. aborted eggs/ female	Emergence/ females %
Control		40	93.8	95.0 / 87.2	18.7 ± 2.8	0.091	100	0.6	90.0 / 44.4
15 / A / 10	-- / -- / --	24	100	100 / 100	15.1 ± 2.3	0.035	37.5	1.5	93.8 / 33.3
20 / A / 20	-- / -- / --	20	100	100 / 92.3	16.0 ± 2.5	0.014	15.6	0.6	3 / 5 / 2 / 3
15 / N / 10	-- / -- / --	44	86.6	88.4 / 83.7	16.8 ± 2.1	0.068	74.8	0.7	94.1 / 53.1
20	-- / -- / --	44	91.6	97.6 / 91.5	13.9 ± 3.6	0.065	71.4	1.0	73.8 / 41.7
40	-- / -- / --	32	18.3	63.6 / 47.6	----	0.007	8.1	1.1	-- / --
20 / N / 20	-- / -- / --	45	97.5	100 / 100	15.9 ± 1.5	0.008	8.3	0.9	5 / 6 / 4 / 5
40	-- / -- / --	40	93.1	95.0 / 95.0	16.4 ± 1.9	0.010	11.4	0.2	5 / 7 / 4 / 5
60	-- / -- / --	40	92.1	100 / 100	16.0 ± 1.4	0.005	4.9	0.2	2 / 3 / 1 / 2
80	-- / -- / --	40	96.1	100 / 100	---	0.000	0.0	0.1	-- / --
15 / N / 10	16 / N / 10	44	91.2	76.1 / 75.6	14.9 ± 2.9	0.056	61.2	1.2	71.7 / 57.6
	30	40	66.8	81.1 / 73.0	11.5 ± 3.7	0.014	14.9	1.0	3 / 9 / 2 / 3
15 / N / 10	17 / N / 10	48	93.7	97.8 / 89.3	14.7 ± 3.4	0.060	65.8	0.8	63.6 / 42.9
	30	42	87.5	73.8 / 73.8	14.1 ± 3.0	0.038	41.4	1.1	55.2 / 75.0
15 / N / 10	20 / N / 10	45	96.4	95.0 / 90.0	----	0.003	2.9	1.0	2 / 2 / 0 / 2
	30	45	93.3	100 / 100	----	0.000	0.0	0.2	-- / --
	50	45	97.8	100 / 100	----	0.003	2.7	0.0	2 / 2 / 1 / 2
	70	45	89.1	100 / 100	----	0.000	0.0	0.0	-- / --

1. Day: Days post larviposition, Atm.: Irradiation atmosphere (A = Air, N = Nitrogen), Dose: Gy
2. Survival relative to mature female days
3. MS +: Mating Scars present
   SP +: *Spermathecae impregnated with motile sperm*
4. No. pupae per mature female day

general biased in favour of males, the differences were not significant (chi square, $p > 0.05$).

For a given radiation dose, the proportion of reproductive abnormalities was comparable for females mated with males irradiated in a single dose on day 15 PL or with doses split 1 day apart. The percent reproductive abnormalities decreased when doses were separated with 2 and 5 days (Fig. 2).

Survival of males treated with doses split in 2 fractions 1 and 2 days apart was slightly better (40 Gy treatment) or resembled (20 Gy treatment) the survival curve of males irradiated on day 15 with a single dose (Fig. 3). Males irradiated in fractions on day 15 and 20 PL survived better for the same radiation dose as compared to males irradiated in one continuous dose on day 15 but had similar survival rates as males treated in one dose on day 20.

Females irradiated with 20 Gy in doses split into fractions had comparable survival rates as females treated with a continuous dose. However, when doses were increased to 40 Gy and administered in one dose on day 15 PL, more than 80% of the females had died on day 40 following emergence. Survival rates increased to 66.8%, 87.5% and 93.3% when the fractions were separated by 1, 2 and 5 days. Receptivity to mating of females treated with 40 Gy in doses 1 and 2 days apart, remained below control level (< 75% inseminated) but all females were found with motile sperm in the spermathecae when doses were separated by 5 days. Splitting the radiation dose in two fractions reduced fecundity of the females slightly (61.2% and 65.8% residual fertility for fractions given 1 and 2 days versus 71.4% when dose was given on day 15 PL). When fractions were separated by 5 days, residual fertility remained below 3%, irrespective of the irradiation dose.

Optimal radiation procedures

Fig. 4 presents the relationship of male fertility (expressed as the number of pupae produced (per mature female day) of their female mates) and survival rate expressed as LT_{50}. The top graph shows that irradiation on day 15 PL in nitrogen resulted in a 25% loss in sterility as compared with irradiation in air, but survival was increased threefold (from LT_{50} of 20 to 68 days). The level of sterility was even more reduced (40% loss) when the irradiation in nitrogen treatment was administered on day 20 PL, but average longevity was increased 4 times. The bottom graph indicates that fractionating the doses was not accompanied with a loss in sterility except when fractions were separated by 5 days. Viability was however considerably increased.

High quality (LT_{50} > 20 days) sterile (residual fertility < 5%) males could be obtained by irradiating pupae in nitrogen during the mid pupal phase in 2 fractions separated by 1 and 2 days (total dose 40 Gy) and 5 days apart (total dose of 60 and 80 Gy).

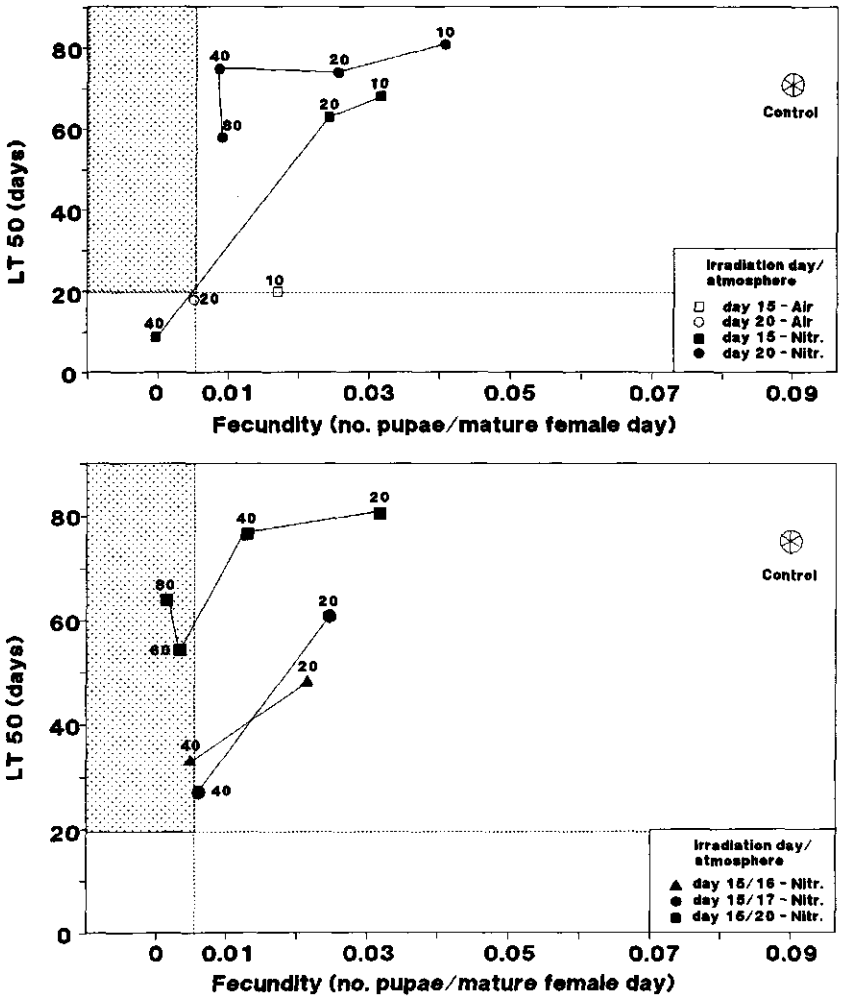

Fig. 4 Fertility of male *G. tachinoides* irradiated as pupae in air and nitrogen atmosphere (top graph) on day 15 and 20 following larviposition and in doses split into two fractions (bottom graph)(1 st dose of 10 Gy was given on day 15 PL; 2 nd dose on day 16, 17 or 20 PL) Figures on graph indicate the irradiation dose in Gy. Shaded area indicates males with a LT_{50} above 20 days and a residual fertility of ≤ 5% of untreated controls.

DISCUSSION

The effect of irradiation in the absence of oxygen on the amount of induced lethal mutations and of somatic injury is very well documented for various insects. The protective effect of nitrogen is related to the absence of hydrogen peroxide, a mutation inducing molecule, which is produced as oxygen reacts with hydrogen atoms originating from water ionised by radiation (Economopolous, 1977). The use of nitrogen during irradiation of *G. tachinoides* pupae in the mid pupal phase (day 15 - 20 following larviposition) reduced sterility levels of adult males by 20 to 40% as compared to irradiation in air and increased their average longevity 3 to 4 times. These results are in agreement with the findings of Curtis & Langley (1972) on *G. m. morsitans* pupae, irradiated in the late pupal phase.

The effect of dose fractionation on male fertility seems dependent on the insect species and the development stage when the fractions are applied. Similar levels of sterility were obtained after exposing male pupae of *Spodoptera littoralis* Boisd. (cotton leaf worm) to X rays in one continuous dose or in various fractions (Wakid *et al.*, 1972). Likewise, exposure of *Ceratitis capitata* (Mediterranean fruit fly) and *Sitophilus granarius* pupae to fractionated doses of gamma radiation resulted in the same level of induced lethal mutations as compared to those obtained after continuous exposure of the same total dose (Jefferies, 1962; Mayas, 1975). Slightly lower levels of induced sterility were obtained with sperm of male *G. m. morsitans* treated in fractionated doses (Dean & Wortham, 1969). Our experiments with *G. tachinoides* pupae treated during the mid pupal phase, indicate that sterility levels are significantly influenced by the timing of the radiation treatment i.e. a treatment given in a single dose on day 15 or split into fractions on day 15, 16 or 17 resulted in similar levels of sterility. All these treatments coincide with the process of spermiogenesis occurring between day 12 and 18 (Itard, 1970). Spermatids have been formed and irradiation in a single dose or in fractions seems not to influence the degree of chromosomal damage. However, when the treatment interval is increased to 5 days, the second dose is administered to sperm reaching maturity. A higher second fraction is then required to obtain the same level of sterility. Moreover, these males showed lower fertility levels as compared to males treated in one continuous dose on day 20 PL indicating a differential radiosensitivity of spermatids and spermatozoa related to age.

Even more significant is the increase in average male longevity when doses are split into 2 or more fractions although some insect species e.g. the adult boll weevil (*Anthonomus grandis)* are exceptional in this respect (Flint *et al.*, 1966). The reduction in somatic injury by dose fractionation is generally attributed to repair mechanisms at the molecular level or replacement of damaged cells during the interval after the first radiation dose (Jefferies, 1962). Our data with *G tachinoides* pupae indicate that cell recovery and consequently male survival is significantly influenced by the timing of treatment, the interval between fractions and the total dose received. Dose fractionation had no significant impact on fly survival with low doses (20 Gy) administered in 1 or 2 day intervals. The increase in survival became more apparent with higher radiation doses and longer intervals. However, cytological studies, analysing the impact of gamma radiation on spermatids, spermatozoa and on the mechanism of cell damage and repair, would be required to illuminate some of our observations.

From a practical point of view, the most meaningful result from our experiments is the observation that *G. tachinoides* pupae can be irradiated during the mid pupal life obtaining sterile males with survival rates comparable with untreated males. This, however, requires irradiating pupae in a nitrogen atmosphere and splitting the dose in at least 2 fractions. (The effect of more fractions has not been studied as Jefferies (1962) observed that the most significant somatic recovery occurs between the first and second fraction.) Although these procedures require certain logistic prerequisites and are more cumbersome than simple radiation treatments in air, more flexibility is allowed in the management of long distance shipment of pupal material. Moreover, various reports have indicated the superior competitiveness of insects treated in nitrogen (Curtis & Langley, 1972) and with doses split into fractions (Mayas, 1975). More in-depth laboratory and field studies on the behaviour and competitiveness of *G. tachinoides* males when treated in the mid pupal phase, could consolidate our observations of their high biological quality and could entitle the procedure as a valid option for application in the field.

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Chapter 5

RADIATION STERILISATION OF *Glossina tachinoides* Westwood PUPAE: III. THE COMBINED EFFECTS OF CHILLING AND GAMMA IRRADIATION

Abstract

Female and male *Glossina tachinoides* Westwood were exposed as 5 day old pupae to 15 °C for 9 to 21 days. Female pupal development was delayed with 10.4 and 18.4 days and male pupal development with 9.9 and 18.4 days for pupae incubated for 9 and 21 days respectively. Pupal eclosion was only affected for chilling periods exceeding 15 days. Mating response, insemination capacity and fertility of males exposed as pupae to a 9 day chilling period was not affected, but their survival was significantly reduced from 52.1 ± 26.2 days to 35.3 ± 18.8 days. Survival of adult females was reduced when exposed as pupae to chilling periods exceeding 12 days. After 9 days of low temperature incubation, females produced however 11% less offspring than untreated females.

Pupae, incubated for 9 days at 15 °C when 5 or 10 days old, were irradiated with 10 and 20 Gy in air or nitrogen 1 hour, 7 hours, 1 day, 3 days and 5 days after the incubation treatment. In general, eclosion rate, male fertility and average male survival was increased when the radiation treatment was given in nitrogen and when chilling and irradiation treatments occurred later in pupal life. Only males incubated for 9 days as 5 day old pupae and irradiated with 10 Gy in air on day 20 PL had a residual fertility below 5% and lived on average longer than 20 days.

Survival of all experimental female flies was reduced as compared to the control. Their receptivity to mating remained however normal in most cases. Complete sterility was induced in females, incubated as 5 day old pupae and irradiated with 10 Gy in air on day 15 - 20 post larviposition and in females, incubated as 10 day old pupae and treated with 10 Gy in air on the 20 th or 21 st day of pupal life.

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INTRODUCTION

Previous radiation research carried out on *Glossina tachinoides* pupae has acknowledged the feasibility of irradiating pupae in air during the late pupal phase without a significant reduction in male fly quality (chapter 3). The brief interval between irradiation and eclosion of the flies remains a constraint when pupae have to be dispatched for long distance transport. It is worthwhile to evaluate manipulation techniques to increase the total time for preparing and transporting pupae i.e. pupae can be irradiated at earlier stages or efforts can be undertaken to arrest pupal development. It was shown in chapter 3, that high quality sterile *G. tachinoides* males can be obtained by treating pupae with ionising radiation during the mid pupal phase (day 15 - 20 PL). This however requires the use of nitrogen during the radiation treatments and the splitting of the radiation dose in at least two fractions.

The major factor influencing the duration of the pupal development is the ambient temperature (Buxton, 1955). The total development period from pupation to emergence can be substantially extended by exposing pupae to low temperatures. Cooling of pupae to inhibit male eclosion after the female eclosion flush, has been used successfully during the SIT pilot trial against *G. m. morsitans* in Tanzania. Storing the male pupae during their late pupal phase at 10 ± 1 °C for 4 days combined with an irradiation treatment in nitrogen did not affect male eclosion (Williamson *et al.*, 1983b) and male quality after release in the field (Williamson *et al.*, 1983d).

This paper presents the results of a study analysing the effects of prolonged periods (9-12 days) of low temperature incubation (15 °C) of *G. tachinoides* pupae and the combined effects with gamma irradiation treatments at ambient temperatures in air and nitrogen. Parameters examined were pupal development period, eclosion, fertility and survival of both male and female flies.

MATERIAL AND METHODS

All *G. tachinoides* pupae used for the experiments were derived from the mass rearing colony maintained at the Entomology Unit of the IAEA 's laboratories in Vienna. Experimental adult male and female flies were maintained under standard holding conditions (22 ± 1°C and 75 ± 5% R.H.) together with untreated controls as described earlier (chapter 3).

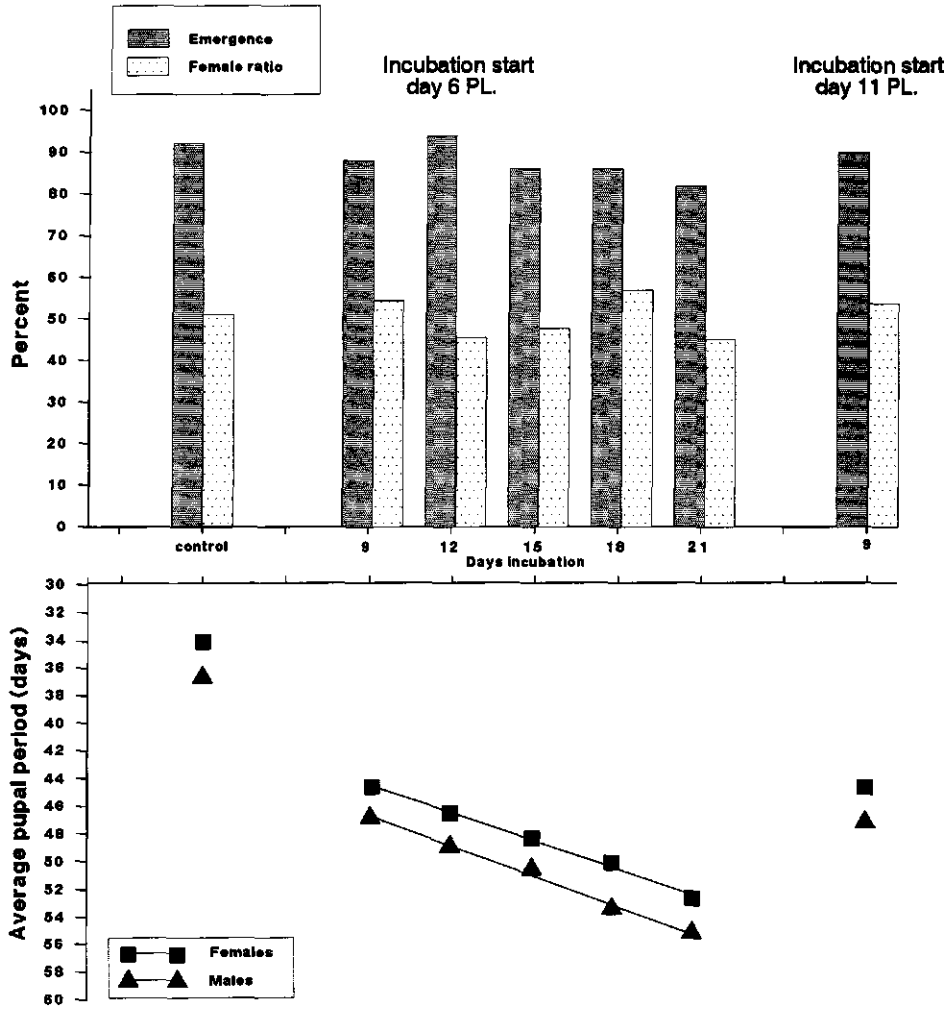

Fig. 1 Eclosion rate and average pupal period of *G. tachinoides* pupae incubated for various periods at 15 °C.

The effects of long term incubation at low temperatures was assessed by exposing batches (n = 100) of 5 day old pupae at 15 ± 1 °C in an incubator for 9, 12, 15, 18 or 21 days and a batch (n = 100) of 10 day old pupae for 9 days. Relative humidity was maintained at 75 ± 5%.

Table 1. Fertility of female and male *G.tachinoides* incubated as pupae at 15 °C for 9 to 21 days and mated with untreated colony flies

Incubation period [1]	Initial females no.	Female survival day 45 % [2]	Mating status MS + / SP + % [3]	Mean puparial weight (mg) ± SD	Fecundity [4]	Production relative to control	Aborted eggs & imm. larvae no.	Emergence/ females %
Control	79	80.8	97.2 / 95.9	17.3 ± 2.8	0.081	100	25	83.2 / 48.2
FEMALE FERTILITY								
6 - 15	40	78.7	97.3 / 94.5	17.1 ± 2.6	0.072	88.2	15	95.2 / 55.9
6 - 18	40	78.8	94.3 / 88.6	15.9 ± 2.8	0.054	67.1	41	83.3 / 45.0
6 - 21	40	38.8	96.3 / 88.8	15.5 ± 2.9	0.048	59.7	10	76.2 / 62.0
6 - 24	40	28.2	96.5 / 71.4	13.3 ± 1.8	0.022	27.4	15	75.0 / 50.0
6 - 27	35	12.6	83.3 / 41.6	------	0.000	0.0	7	----
11 - 20	40	80.1	94.4 / 91.6	15.7 ± 2.3	0.072	88.8	28	95.2 / 44.1
MALE FERTILITY								
6 - 15	40	88.8	97.4 / 97.4	16.5 ± 2.7	0.083	101.7	18	91.3 / 50.0
6 - 18	40	86.6	100 / 88.8	16.1 ± 2.7	0.072	89.0	30	91.4 / 54.7
6 - 21	35	88.9	100 / 66.6	17.9 ± 2.0	0.048	59.4	57	95.2 / 67.5
6 - 24	30	83.9	81.5 / 37.0	16.5 ± 4.1	0.023	28.1	68	87.5 / 78.5
6 - 27	30	82.9	93.3 / 30.0	16.6 ± 2.1	0.022	26.6	71	93.3 / 55.5
11 - 20	40	84.0	97.3 / 97.3	17.8 ± 3.1	0.098	120.6	17	92.1 / 54.8

1. Days post larviposition
2. Survival relative to mature female days
3. MS +: Mating Scars present
   SP +: Spermathecae impregnated with motile sperm
4. No. pupae per mature female day

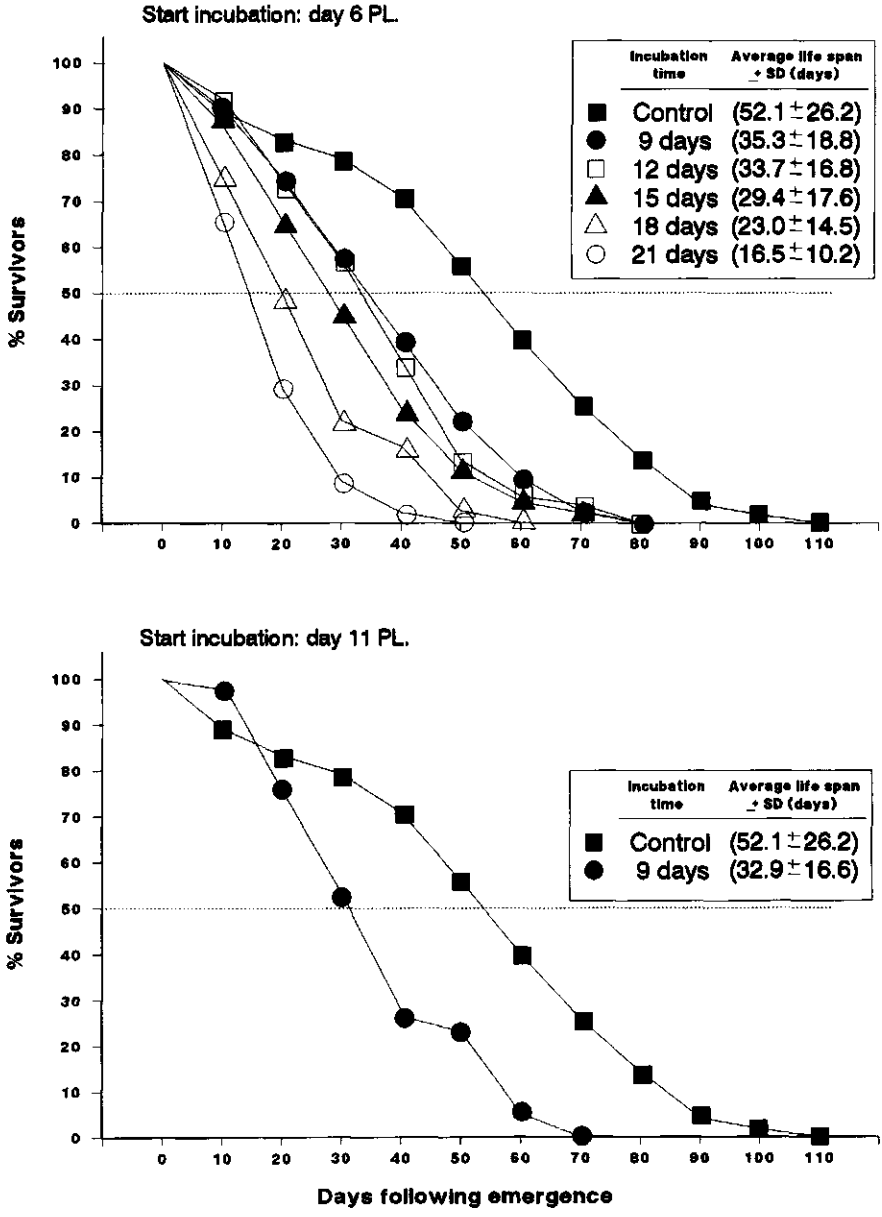

Fig 2. Survival curves of *G. tachinoides* males incubated at 15°C for various periods starting on day 6 (top) and day 11 (bottom) PL.

Afterwards, all experimental pupae were transferred to the insectary to complete their development under standard holding conditions.

For the assessment of the combined effects of chilling and irradiation, batches (n = 100) of 5 day old (INCUBATION GROUP I) and 10 day old (INCUBATION GROUP II) pupae were incubated for 9 days at 15 ± 1 °C prior to the irradiation treatments. Pupae were irradiated with doses of 10 and 20 Gy in a ^{60}Co source (dose rate 6 Gy/min.) 1 hour, 7-8 hours, 1 day, 3 days and 5 days respectively after incubation. Irradiation in nitrogen atmosphere was carried out as described in a previous paper (chapter 4), whereas collection of emerging flies and experimental procedures to assess reproductive parameters were used as described in chapter 2.

RESULTS

EFFECTS OF LOW TEMPERATURE (15 °C) INCUBATION

Eclosion rate and average pupal period of female and male *G tachinoides*, incubated at 15 °C as 5 day old pupae for 9 to 21 days and as 10 day old pupae for 9 days are presented in Fig. 1. An average pupal period of 34.2 ± 0.5 and 36.9 ± 0.6 days was recorded for female and male pupae respectively under the prevailing standard holding conditions. The 9 day incubation period delayed female and male development with 10.4 and 9.9 days respectively whereas female and male pupae exposed to 15 °C for 21 days required 52.6 ± 0.6 days and 55.3 ± 0.7 days for completion of their development. A difference in average development period of 1.04 days was recorded between female and male pupae incubated for 9 - 15 days. This sex related difference was comparable to the one observed for untreated pupae (1.07 days) but was increased to 2.7 and 3.1 days for longer incubation periods. Adult eclosion was not adversely affected by cooling periods up to 12 days (88% - 94% eclosion versus 92% for control pupae (chi square, $p > 0.05$)), but longer incubation periods reduced fly eclosion significantly to 82 - 86% (chi square, $p < 0.01$).

The mating response of all experimental adult males was normal with > 81% of the female mates showing mating scars. Male insemination capacity however, dropped from 97.4% to 89% and 66.6 - 30% for males exposed to 15 °C as 5 day old pupae for 9, 12 and 15 - 21 days respectively. Sperm quality of males exposed for 9 days as 5 and 10 day old pupae was not affected as evidenced by a fertility of 0.083 and 0.098 pupae per mature female day respectively (control

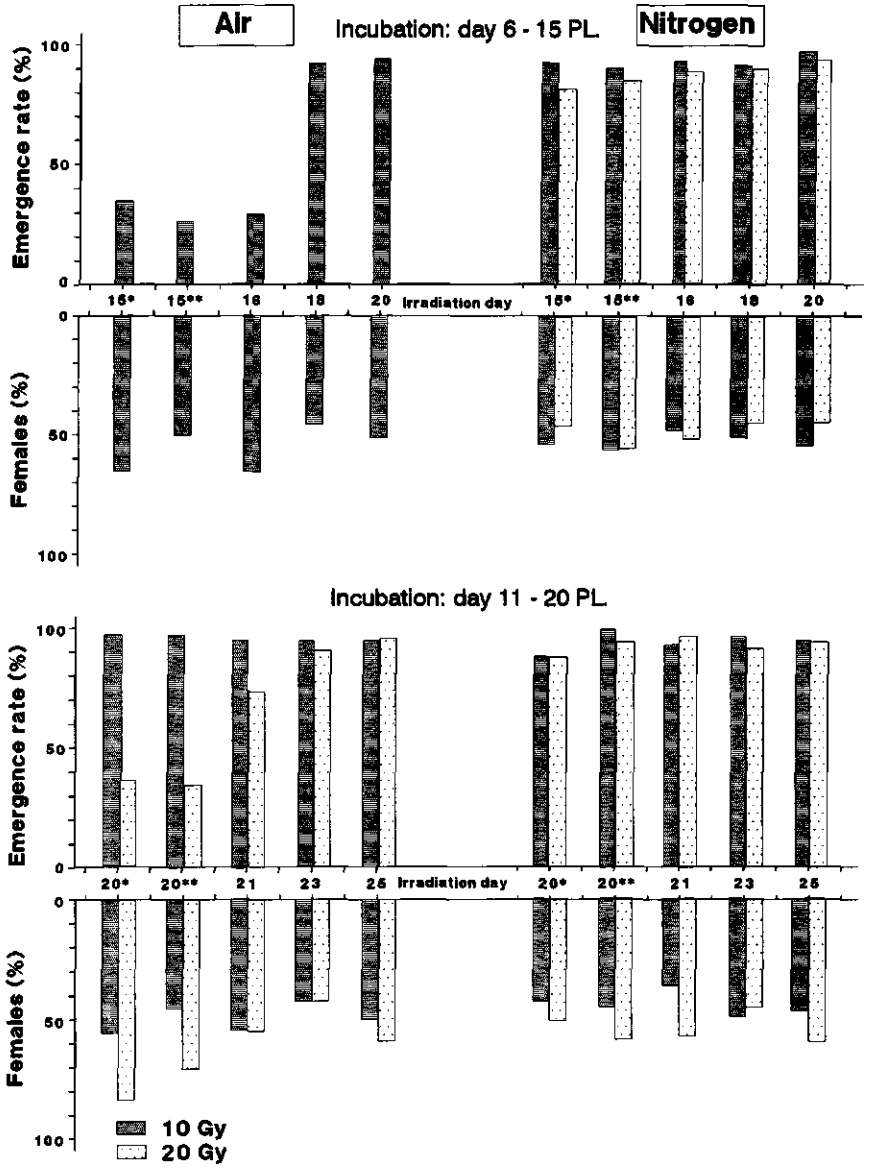

Fig. 3 Eclosion rate and female ratio of *G. tachinoides* pupae incubated from day 6 to 15 PL (top) and from day 11 to 20 PL (bottom) and irradiated with 10 and 20 Gy in air or in nitrogen atmosphere. (* irradiated treatment 1 hour and **7 - 8 hours after incubation)

females produced 0.081 pupae/mature female day) (Table 1). Cooling male pupae for 12 days reduced their fertility with 11% as compared to untreated males. Longer cooling periods reduced male fertilities proportionally with the length of incubation. These data were corroborated by the dissection data of the female flies. The proportion of inseminated females showing aberrations in the ovarian configuration and uterus content did not exceed 3% for females mated with males incubated for 9 days. This percentage of females displaying reproductive abnormalities increased to 4.8% - 13.8% for females mated with males incubated for longer periods. Viability of offspring was however normal (eclosion rate > 87%) with sex ratios slightly biased in favour of females (only significant for 15 day incubation group (chi square = 4.22, $p < 0.05$)).

Unchilled control males lived on average 52.1 ± 26.2 days (Fig. 2). Incubation of 5 and 10 day old male pupae for 9 days reduced the average longevity of the adult males significantly ($p < 0.01$) with 16.8 and 19.2 days respectively. Average life spans of adult males were proportionally decreased when incubated as pupae for longer periods. An average life span of 16.5 ± 10.2 days was recorded for males incubated as pupae for 21 days.

Untreated female flies and females incubated as 5 and 10 day old pupae for 9 days and 5 day old pupae for 12 days showed comparable survival rates during the 45 day experimental period (Table 1). Increasing the incubation period to 15, 18 and 21 days reduced female survival significantly (38.8%, 28.2% and 12.6% survivors respectively on day 45). Although more than 83% of all experimental females were receptive to mating, insemination rate dropped below 72% for females incubated for more than 18 days. Reproduction rate of all experimental females was inferior to reproduction of untreated controls. No pupae were produced by females incubated as pupae for 21 days.

COMBINED EFFECTS OF LOW TEMPERATURE (15 °C) INCUBATION AND GAMMA IRRADIATION IN AIR AND NITROGEN

Pupal development and eclosion

Eclosion data of pupae incubated from day 6 to day 15 PL (Incubation group I) and from day 11 to day 20 PL (Incubation group II) and irradiated with 10 and 20 Gy in air and nitrogen atmosphere are presented in Fig. 3. Irradiating pupae of incubation group I with 10 Gy in

Table 2a. Fertility of male *G. tachinoides*, incubated as pupae at 15 °C for 9 days (day 6 - 15 PL.) and irradiated with 10 - 20 Gy in air or in nitrogen atmosphere and mated with untreated colony females

Irradiation atm./dose/day (A - N) (Gy) (PL.) [1]	Initial females no.	Female survival day 45 %[2]	Mating status MS + / SP + % [3]	Mean puparial weight (mg) ± SD	Fecundity [4]	Production relative to control	No. aborted eggs	Emergence/ females %
Control	79	80.8	97.2 / 95.9	17.3 ± 2.8	0.081	100	20	83.2 / 48.2
A / 10 / 18	10	91.8	90.0 / 90.0	------	0.008	9.6	36	2 / 2 / 1 / 2
/ 20	30	78.8	92.0 / 84.0	------	0.000	0.0	97	-- / --
N / 10 / 15 *	18	91.9	94.4 / 88.8	14.0 ± 3.0	0.041	50.6	42	100 / 52.4
/ 15 **	20	98.2	90.0 / 80.0	13.9 ± 2.6	0.056	69.5	30	96.9 / 59.4
/ 16	30	90.5	92.6 / 96.3	13.6 ± 2.3	0.050	61.7	43	92.8 / 51.3
/ 18	20	79.5	100 / 94.4	13.1 ± 2.5	0.027	33.3	45	100 / 41.2
/ 20	20	87.0	100 / 100	15.3 ± 2.8	0.027	32.9	45	88.2 / 33.3
N / 20 / 20	28	58.3	90.9 / 68.2	-----	0.002	2.7	63	1 / 1 / 1 / 1

1. PL: Days post larviposition
   A: Air
   N: Nitrogen
   (*) Irradiation 1 hour and (**) 7 - 8 hours after incubation
2. Survival relative to mature female days
3. MS +: Mating Scars present
   SP +: Spermathecae impregnated with motile sperm
4. No. pupae per mature female day

Table 2b. Fertility of male *G. tachinoides*, incubated as pupae at 15 °C for 9 days (day 11 - 20 PL.) and irradiated with 10 - 20 Gy in air or in nitrogen atmosphere and mated with untreated colony females

Irradiation atm./dose/day (A - N) (Gy) (PL.) [1]	Initial females no.	Female survival day 45 %[2]	Mating status MS + / SP + % [3]	Mean puparial weight (mg) ± SD	Fecundity [4]	Production relative to control	No. aborted eggs	Emergence/ females %
Control	79	80.8	97.2 / 95.9	17.3 ± 2.8	0.081	100	20	83.2 / 48.2
A / 10 / 20 *	20	90.0	88.8 / 88.8	------	0.004	4.9	74	0 / 2 / 0 / 0
/ 20 **	30	98.1	96.4 / 92.8	------	0.001	1.5	120	1 / 1 / 1 / 1
/ 21	30	89.4	93.1 / 93.1	16.0 ± 3.3	0.008	9.9	104	6 / 6 / 3 / 6
/ 23	35	83.1	96.6 / 90.0	16.1 ± 3.1	0.012	15.2	110	6 /10 / 3 / 6
/ 25	35	94.3	97.0 / 94.1	16.5 ± 2.7	0.017	21.3	114	92.9 / 30.8
N / 10 / 20 *	30	87.6	100 / 100	16.3 ± 2.6	0.049	60.3	50	82.8 / 37.9
/ 20 **	30	88.7	100 / 100	16.4 ± 3.0	0.048	59.6	50	88.8 / 62.5
/ 21	30	97.3	100 / 93.3	17.8 ± 2.4	0.032	39.2	72	80.7 / 47.6
/ 23	30	91.8	100 / 100	17.0 ± 2.7	0.048	59.2	58	86.1 / 41.9
/ 25	36	99.3	97.2 / 97.2	16.8 ± 2.9	0.051	62.8	75	84.0 / 76.2
N / 20 / 20 *	25	82.1	95.2 / 85.7	17.1 ± 2.4	0.021	25.8	59	83.3 / 50.0
/ 20 **	25	87.1	82.6 / 91.3	16.2 ± 3.0	0.016	20.2	71	90.0 / 44.4
/ 21	30	87.6	92.6 / 88.8	15.6 ± 2.6	0.026	31.8	69	78.9 / 20.0
/ 23	30	96.7	90.0 / 83.3	16.8 ± 3.0	0.022	27.1	94	82.4 / 35.7
/ 25	30	85.5	92.0 / 92.0	17.0 ± 2.5	0.025	30.9	76	77.8 / 57.1

1. PL: Days post larviposition
   A: Air
   N: Nitrogen
   (*) Irradiation 1 hour and (**) 7 - 8 hours after incubation
2. Survival relative to mature female days
3. MS +: Mating Scars present
   SP +: Spermathecae impregnated with motile sperm
4. No. pupae per mature female day

air on day 15 and 16 PL reduced eclosion significantly to < 34% (chi square, $p < 0.01$). Examination of the content of the uneclosed pupae revealed that both males and females completed their development but failed to emerge. Eclosion rate was increased to the control level of > 92% when the same irradiating treatment was given on day 18 - 20 PL. All pupae were killed with a treatment of 20 Gy in air. The use of nitrogen during the 10 and 20 Gy radiation treatment resulted in optimal eclosion rates (> 90%) except for pupae treated with 20 Gy on day 15 - 16 PL (81% (chi square, $p < 0.01$) and 88% (chi square , $p < 0.05$)).

More than 87% of the pupae emerged when incubated from day 11 to 20 PL prior to an irradiation treatment of 10 Gy in air and 10 - 20 Gy in nitrogen (chi square, $p > 0.05$). An irradiation dose of 20 Gy applied in air on day 20 reduced the eclosion rate to < 36% (chi square, $p < 0.01$). Female and male pupae were not killed by the irradiation treatment and continued their development to completion but failed to rupture the puparium. The same radiation treatment applied 3 to 5 days after the incubation, resulted in a normal pupal development and 90% eclosion.

Adult male fertility and survival

All experimental adult males, exposed as pupae to 9 days of cooling followed by an irradiation treatment showed a normal mating response (> 82% of the female mates showed mating scars), spermatophore formation and sperm transfer (> 80% of the females inseminated). Aberrant results were observed for males of the first incubation group, treated with 20 Gy on day 20 PL in nitrogen who failed to inseminate 32% of their female mates.

All emerged males from the first incubation group, treated as 15 - 16 day old pupae with 10 Gy in air died before reaching sexual maturity (Table 2a-b). Nor were sufficient sexually mature males available for assessment of fertility parameters, having emerged from pupae treated with 20 Gy in nitrogen on day 15 - 18 PL The use of nitrogen during irradiation increased adult male fertility significantly (threefold) as compared to treatments given in air. Adult male fertility was dependent on the age of the pupae when treated with 10 Gy in nitrogen i.e. fertility decreased from 0.041 - 0.056 to 0.027 pupae/mature female for males treated as 15 - 16 day old pupae and 18 - 20 day old pupae respectively. More than 95% sterility was induced in the sperm of adult males when treated on day 20 PL with 10 Gy in air and 20 Gy in nitrogen. Although all pupae produced by females mated with irradiated

Table 3. *Average longevity of G.tachinoides males incubated for 9 days at 15 °C. and irradiated in air and nitrogen with 10-20 Gy during various moments of their pupal development*

Irradiation Atm./dose/day (A/N) (Gy) (PL)		Males no.	Average life span ± SD (days)	Survival (%) on indicated days following emergence					
				10	20	30	40	50	60
Control									
– – –		80	52.1 ± 26.2	89.2	83.3	79.2	70.8	55.8	40.0
Incubation period : day 6 - 15 PL.									
– – –		40	35.3 ± 18.8	80.0	75.0	57.5	40.0	22.5	10.0
A / 10 / 15	*	12	3.0 ± 0.3	0.0	0.0	0.0	0.0	0.0	0.0
15	**	13	3.2 ± 0.5	0.0	0.0	0.0	0.0	0.0	0.0
16		10	3.7 ± 2.2	0.0	0.0	0.0	0.0	0.0	0.0
18		50	8.8 ± 9.9	24.0	16.0	6.0	2.0	0.0	0.0
20		46	24.4 ± 21.2	71.7	43.5	32.6	17.4	13.0	10.9
N / 10 / 15	*	40	17.0 ± 17.2	55.0	22.5	20.0	15.0	5.0	2.5
15	**	40	14.4 ± 17.3	47.5	20.0	15.0	15.0	7.5	5.0
16		49	24.4 ± 26.3	61.2	34.7	24.5	24.5	20.4	16.3
18		43	17.0 ± 31.0	51.5	20.0	15.6	15.6	13.3	6.7
20		39	26.7 ± 32.2	59.0	38.5	33.3	20.5	15.4	15.4
N / 20 / 15	**	44	3.8 ± 2.0	2.3	0.0	0.0	0.0	0.0	0.0
15	*	38	5.2 ± 3.3	18.4	0.0	0.0	0.0	0.0	0.0
16		43	5.0 ± 4.1	16.3	0.0	0.0	0.0	0.0	0.0
18		50	5.9 ± 3.3	18.0	0.0	0.0	0.0	0.0	0.0
20		51	11.0 ± 9.1	53.8	11.5	3.8	1.9	1.9	0.0
Incubation period: day 11 - 20 PL.									
– – –		41	32.9 ± 16.6	97.6	75.6	53.7	26.8	22.0	4.9
A / 10 / 20	*	42	18.4 ± 17.0	54.8	40.5	7.1	7.1	7.1	4.8
20	**	51	18.0 ± 14.7	56.9	35.5	15.7	9.8	5.9	3.9
21		41	20.8 ± 16.2	65.9	34.1	24.4	12.2	12.2	2.4
23		47	17.4 ± 10.0	72.3	40.4	10.6	2.1	0.0	0.0
25		47	20.3 ± 16.5	76.6	25.5	12.8	12.8	12.8	4.3
A / 20 / 20	*	6	4.0 ± 3.0	0.0	0.0	0.0	0.0	0.0	0.0
20	**	10	2.2 ± 0.8	0.0	0.0	0.0	0.0	0.0	0.0
21		33	2.8 ± 1.1	0.0	0.0	0.0	0.0	0.0	0.0
23		51	4.3 ± 1.6	0.0	0.0	0.0	0.0	0.0	0.0
25		39	8.3 ± 6.3	15.4	5.1	2.6	0.0	0.0	0.0
N / 10 / 20	*	50	19.8 ± 19.3	58.0	30.0	20.0	19.0	6.0	4.0
20	**	53	19.0 ± 14.9	58.5	35.8	22.6	13.2	1.9	1.9
21		56	14.3 ± 12.2	51.8	17.9	10.7	10.7	1.8	0.0
23		48	20.6 ± 18.0	57.1	36.7	30.6	22.4	6.1	4.1
25		51	22.7 ± 18.8	70.6	39.2	23.5	21.6	7.8	7.8
N / 20 / 20	*	43	14.9 ± 13.7	60.5	25.6	14.0	4.7	4.7	0.0
20	**	39	12.7 ± 10.6	64.1	7.7	5.1	5.1	2.6	0.0
21		41	19.9 ± 18.4	75.6	29.3	22.0	14.6	12.2	2.4
23		48	15.2 ± 14.1	63.3	22.4	18.4	10.2	4.1	0.0
25		42	15.5 ± 16.2	69.0	14.3	11.9	11.9	9.5	2.4

* Irradiation 1 hour after incubation

** Irradiation 7 - 8 hours after incubation

males weighed significantly less than pupae fathered by control males ($p < 0.05$), their viability was found to be normal ($p > 0.05$).

Fertility of males of the second incubation group, irradiated in air, was increased with increasing pupal treatment age. No such age dependent relation was found when pupae were irradiated in nitrogen. The protective effect of nitrogen on the amount of induced lethal mutations was more pronounced with younger pupae i.e. residual fertilities increased 12 - 48 times with a dose of 10 Gy on day 20 PL versus the same treatment in air but resulted in a 3-4 fold fertility increase only when administered on day 23 - 25 PL. 95% sterility was induced only in males treated with 10 Gy in air on the 20 th day of their pupal life. All treatments in nitrogen resulted in male residual fertility levels exceeding 20% of that of the control.

The proportion of females displaying reproductive abnormalities i.e. uterus empty due to expulsion of a dead embryo or an egg *in utero* in embryonic arrest, increased with increasing radiation dose but decreased when nitrogen was used during irradiation. Between 25 to 52% of the females mated with males belonging to the first incubation group and treated with 10 Gy in air, revealed reproductive abnormalities. This proportion of females decreased to 8.3 - 23% when the same dose was administered in nitrogen, but increased to > 88% when the treatment dose was 20 Gy. The same trend was observed in females mated with males belonging to the second incubation group.

Table 3 presents data on survival of untreated and experimental males. Cooling of pupae followed by irradiation reduced the mean longevity of adult males significantly ($p < 0.05$) more than exposure to a low temperature alone. In general, average male longevity was less severely affected when the radiation treatment was given in nitrogen and when the chilling and irradiation treatments occurred later in pupal life. Only males (incubation group I) treated with 10 Gy in air and nitrogen on day 20 PL and (incubation group II) treated with 10 Gy in air on day 21 and 25 PL and with 10 Gy in nitrogen on day 23 - 25 PL lived on average > 20 days. Consequently, combined survival and fertility data show that only males incubated for 9 days as 5 day old pupae and irradiated with 10 Gy in air on day 20 PL had a residual fertility < 5% of the control and an average longevity above 20 days. None of the males irradiated as pupae in nitrogen could meet these criteria.

Adult female fertility and survival

Survival of all experimental females was reduced (< 67% and < 71% survivors on day 45 for females of incubation group I and II

respectively) compared to survival of untreated females (Table 4a-b). Female survival increased in general with a lower irradiation dose and when administered in nitrogen and during later pupal stages. Their receptivity to mating however remained unaffected except for females of incubation group I treated with 10 Gy in air or with 20 Gy in nitrogen (75 - 84% spermathecae impregnated with sperm).

Females treated as 15 - 20 day old pupae (incubation group I) with 10 Gy in air failed to produce any offspring. Treating female pupae with 10 Gy in nitrogen revealed reproduction rates of 69 - 84% to that of the control but the residual fertility decreased to 15 - 51% with a radiation dose of 20 Gy.

Complete sterility was induced in females of incubation group II and treated with 10 Gy in air on the 20 th or 21 st day of pupal life. The same treatment given on day 23 - 25 PL resulted in residual fertilities of 30 - 55.5%. The use of nitrogen during irradiation, increased residual fertility to 64 - 84%. A dose of 20 Gy in nitrogen resulted in residual fertilities of 19 - 75% depending on the age of pupae during treatment. All pupae produced by the experimental females weighed significantly less in comparison with pupae produced by untreated females ($p < 0.01$). Viability however was unaffected except for offspring produced by females of incubation group I and treated with 20 Gy in nitrogen on day 15 - 18 (chi square, $p < 0.01$) and by females of incubation group II and treated with 20 Gy in nitrogen on day 20 - 23 (eclosion rates < 54.1%, $p < 0.01$).

DISCUSSION

Buxton & Lewis (1934) and Buxton (1955) have shown that the duration of the pupal development period of tsetse flies can be significantly shortened by exposing pupae to temperatures exceeding the optimum of 23 - 24 °C. In addition, recent research has indicated the feasibility of extending the total pupal development period by cooling tsetse pupae for long periods at young stages. Exposing 4 day old *G. austeni* pupae for 9 - 15 days at 15 °C (Feldmann *et al.*, 1992) and 5 - 20 day old *G. p. palpalis* pupae for 5 days at 15 °C (Feldmann & Vreysen, unpublished data) had no adverse effects on total pupal eclosion rate and male insemination capacity. During the entire pupal development period, fat constitutes the sole source of energy (Buxton & Lewis, 1934) with the rate of metabolism being greatest at the start and end of the pupal period (Bursell, 1958). The importance of the fat metabolism in tsetse pupae is underlined by the fact that newly emerged teneral flies have to

Table 4a. Fertility of female *G. tachinoides*, incubated as pupae at 15 °C. for 9 days (day 6 - 15 PL.) and irradiated with 10 - 20 Gy in air or nitrogen atmosphere and mated with untreated colony males

Irradiation atm./dose/day (A/N) (Gy) (PL.)[1]	Initial females no.	Female survival day 45 % [2]	Mating status MS + / SP + % [3]	Mean puparial weight (mg) ± SD	Fecundity [4]	Production relative to control	No. aborted eggs/ female	Emergence/ females %
Control	79	80.8	97.2 / 95.9	17.3 ± 2.8	0.081	100	0.29	83.2 / 48.2
A / 10 / 15 *	21	9.5	-----	------	0.000	0.0	1.00	-- --
/ 15 **	13	0.0	-----	------	0.000	0.0	0.00	-- --
16	16	15.8	-----	------	0.000	0.0	1.00	-- --
18	40	23.1	83.3 / 75.0	------	0.000	0.0	1.92	-- --
20	45	31.8	76.5 / 76.5	------	0.000	0.0	1.19	-- --
N / 10 / 15 *	40	46.0	100 / 90.9	14.0 ± 3.0	0.056	69.4	0.78	87.5 / 50.0
/ 15 **	47	45.4	100 / 96.3	13.9 ± 2.6	0.059	72.4	0.69	91.8 / 52.9
/ 16	37	41.4	100 / 95.4	13.6 ± 2.3	0.068	83.4	0.50	84.3 / 55.6
/ 18	38	36.4	100 / 94.7	13.1 ± 2.5	0.057	70.0	0.26	84.0 / 61.9
/ 20	53	67.9	97.7 / 90.9	15.3 ± 2.8	0.069	84.5	0.67	84.6 / 48.5
N / 20 / 15 *	36	21.4	100 / 76.5	8.9 ± 1.5	0.023	28.5	0.92	1 / 5 / 0 / 1
15 **	45	13.1	100 / 81.8	9.4 ± 3.8	0.012	14.9	0.45	1 / 2 / 1 / 1
16	45	31.5	95.8 / 75.0	9.5 ± 1.6	0.028	34.2	1.06	4 /11 / 2 / 4
18	38	33.2	84.2 / 78.9	6.7 ± 0.9	0.014	17.5	1.47	0 / 5 / 0 / 0
20	40	62.4	93.7 / 84.4	12.2 ± 2.9	0.042	51.2	1.52	75.0 / 61.9

1. PL: Days post larviposition
   A: Air
   N: Nitrogen
   (*) Irradiation 1 hour and (**) 7 - 8 hours after incubation
2. Survival relative to mature female days
3. MS +: Mating Scars present
   SP +: Spermathecae impregnated with motile sperm
4. No. pupae per mature female day

Table 4b. Fertility of female *G. tachinoides*, incubated as pupae at 15 °C for 9 days (day 11 - 20 PL.) and irradiated with 10 - 20 Gy in air or nitrogen atmosphere and mated with untreated colony males

Irradiation atm./dose/day (A/N) (Gy) (PL.)[1]	Initial females no.	Female survival day 45 % [2]	Mating status MS + / SP + % [3]	Mean puparial weight (mg) ± SD	Fecundity [4]	Production relative to control	No. aborted eggs/ female	Emergence/ females %
Control	79	80.8	97.2 / 95.9	17.3 ± 2.8	0.081	100	0.29	83.2 / 48.2
A / 10 / 20 *	40	71.4	100 / 93.5	------	0.000	0.0	0.85	– / –
/ 20 **	35	60.0	96.3 / 96.3	------	0.000	0.0	0.91	– / –
/ 21	45	71.3	95.2 / 92.8	------	0.000	0.0	1.24	– / –
/ 23	33	67.3	96.4 / 92.8	15.2 ± 2.7	0.045	55.5	0.92	80.0 / 40.0
/ 25	40	67.9	93.1 / 89.6	14.7 ± 3.9	0.025	30.8	1.42	76.5 / 46.2
N / 10 / 20 *	35	49.2	100 / 100	14.6 ± 3.5	0.069	84.5	0.36	78.1 / 72.0
/ 20 **	40	39.2	100 / 100	12.9 ± 3.2	0.052	64.6	0.45	72.7 / 37.5
/ 21	33	17.1	100 / 100	14.2 ± 1.6	0.057	70.3	0.71	100 / 55.6
/ 23	45	45.2	100 / 100	16.0 ± 2.5	0.068	84.3	0.46	97.4 / 47.4
/ 25	40	41.2	100 / 100	14.9 ± 3.1	0.056	69.5	0.60	75.0 / 80.0
N / 20 / 20 *	42	21.8	100 / 92.3	10.9 ± 2.4	0.016	19.2	0.61	1 / 4 / 1 / 1
/ 20 **	45	27.9	100 / 88.0	7.0 ± 2.8	0.026	31.6	0.89	1 / 9 / 0 / 1
/ 21	52	43.8	96.9 / 96.0	8.3 ± 1.7	0.028	34.8	0.74	22.2 / 3 / 4
/ 23	37	44.4	100 / 86.4	15.2 ± 3.7	0.061	75.1	0.74	54.1 / 53.8
/ 25	54	33.1	100 / 96.7	13.2 ± 3.1	0.038	46.9	0.81	66.6 / 41.6

1. PL: Days post larviposition
   A: Air
   N: Nitrogen
   (*) Irradiation 1 hour and (**) 7 - 8 hours after incubation
2. Survival relative to mature female days
3. MS +: Mating Scars present
   SP +: Spermathecae impregnated with motile sperm
4. No. pupae per mature female day

rely solely on their residual fat content for energy until their first blood meal. Moreover, fat consumption in tsetse pupae is greatly influenced by the ambient temperature. Bursell (1960b) showed that consumption of fat in *G. m. morsitans* pupae was most economic at 24 °C and increased significantly above and below this optimum (Phelps, 1973). Although inter-specific differences in rate of fat consumption have been demonstrated (Langley, 1971), the total amount of fat reserves of a small tsetse species as *G. tachinoides* is far less as compared with larger species. Consequently, smaller individuals exposed to low temperatures during their pupal development, will reach critical levels of their total reserves of fat sooner as compared with larger ones. Our data with *G. tachinoides* pupae indicate that chilling pupae for 9 - 12 days at 15 °C did not deplete their total fat content and male and female pupal development could be completed. Extending the incubation period beyond 15 days resulted in a 10% increase in pupal death. However, a 9 day chilling period seemed to be the upper threshold in terms of male insemination capacity and fertility. Average male survival was however significantly reduced. Moreover, female flies exposed as pupae to the 9 day cooling treatment, had similar survival rates as untreated females but produced 11% less offspring. These results strongly suggest that viability of adult male and female *G. tachinoides* flies is affected by factors other than pupal fat metabolism when exposed as pupae to extended periods of low temperatures. In addition, a study to reveal the mechanisms responsible for the reduced fertilities in both male and female flies would certainly be a challenging research topic.

The feasibility of chilling late pupal stage *G. m. morsitans* pupae followed by a sterilising radiation dose has been demonstrated by Curtis & Langley (1972). A cooling period of 5 days at 10 °C combined with a sterilising treatment in nitrogen after the incubation, could effectively control male emergence without a loss of adult male quality. Prolonging the cooling period to 7 - 10 days resulted however in 10% pupal death (Langley *et al.*, 1974). Likewise, viability of adult male *Ceratitis capitata*, exposed as pupae for 2 hours or 2 days to 5 °C or 15 °C prior to irradiation, was not significantly affected. The chilling treatment however, reduced sterility as compared with pupae incubated at 25 °C (Wakid *et al.*, 1982). Cooling *Ceratitis capitata* pupae during irradiation i.e. reducing the metabolic rate, did in addition not give any protection due to the increased solubility of oxygen in the tissues (Langley & Maly, 1971). Our experiments with *G. tachinoides* pupae have shown that combining chilling with low dose irradiation treatment at ambient temperatures increased the loss in viability of

adult males already depicted by a cooling treatment alone. The use of nitrogen during irradiation reduced the amount of somatic injury expressed by an increase in mean longevity but was accompanied by a reduction in the amount of induced lethal mutations. These data are in accordance with observations made by Curtis & Langley (1972) and in this thesis (Chapter 4).

In conclusion, exposing young *G. tachinoides* pupae to low temperatures followed by a sterilising irradiation treatment seems to leave only limited possibilities for the manipulation required for an extended handling period. Sufficient sterility was induced in male adult tsetse flies exposed to a 9 day incubation period (15 °C) starting on the 6 th day of their pupal development, followed by a 10 Gy irradiation treatment in air on day 20 PL. Although the treatment created a 25 day interval between irradiation and male eclosion, average survival of the adult males was reduced to 24 days. These survival data are comparable with the ones obtained by treating *G. tachinoides* pupae in air with the sterilising dose of 20 Gy on day 20 PL (chapter 3). The interval between treatment and onset of male eclosion remained however limited to 12 - 14 days. Higher quality sterile males in terms of survival (mean longevity > 30 days) were obtained by irradiating unchilled 15 - 20 day old pupae in nitrogen in doses split in two fractions. An interval of 12 to 16 days between treatment and male eclosion was obtained depending on the treatment option (chapter 4).

This series of experiments amply demonstrated the feasibility of producing high quality sterile males by irradiation of *G. tachinoides* pupae aged between 15 to 20 days. In addition, the exposure of young pupae to low temperatures significantly prolonged the pupal period, but the viability of the adult males was negatively affected when combined with a sterilising treatment. Further research should be carried out to improve the quality of the obtained males. Splitting the radiation dose in two fractions after the incubation period is certainly one of the options that should be investigated.

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Chapter 6

THE EFFECT OF IONISING RADIATION ON THE REPRODUCTIVE BIOLOGY OF *Glossina austeni* Newstead FEMALES

Abstract

A 60 Gy gamma treatment administered to female *Glossina austeni* on day 2 or 9 following emergence, and likewise, a 50 Gy gamma treatment given to pupae on day 33 following larviposition, induced complete sterility in the female flies without altering their mating behaviour. Treated females remained receptive to mating with untreated males up to 15 days following emergence (mating response of 84%).

The timing of treatment (on day 33 post larviposition, on day 2 and 9 following emergence) influenced significantly the dynamics of the follicle development. Females, irrespective of their age when treatment was received, showed a normal development pattern of the follicles in position A_1 and C_1 i.e. normal vitellogenesis, maturation and ovulation. Females treated as pupae however, revealed no visible signs of a development of follicles in position B_1 and D_1. From day 15 on, females displayed inactive ovaries characterised by atrophied oocytes and nurse cells. Treating females on day 2 or 9 of their adult life, resulted in various degrees of development of the B_1 and D_1 follicles.

During laboratory cage tests, untreated males exposed to equal numbers of virgin untreated and treated females, showed no significant preference to mating with either type of female.

A high degree of multiple mating was observed when untreated and treated females were offered several mating opportunities. On day 9 following emergence, 24.0% of untreated and 23.8% of females treated with 60 Gy (on day 2), accepted a male during a 4 th mating occasion. The receptivity to remating decreased with a higher radiation dose (120 Gy) and when treatment was given later in the female life (day 5).

The results of the laboratory experiments are discussed in view of deploying gamma sterilised female *G. austeni* for entomological monitoring in those areas where low fly densities exist and especially, to expose potential relic fly pockets after control operations have been completed.

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INTRODUCTION

Entomological monitoring using biological evaluation systems, provides essential information during tsetse control and eradication operations on the degree of control achieved at any given time. Monitoring and consequently the successful planning of a campaign depends primarily on the reliability and efficiency of the deployed monitoring device. Serious problems are experienced by tsetse control officers when the wild fly population drops below the threshold level of detection of the used trapping device. Therefore, the release-recapture of gamma sterilised female tsetse flies was postulated (Van der Vloedt & Barnor, 1984) as an efficient method for the detection of low density fly populations and especially, at the end of the control programme, to confirm the status of eradication. Evidence for mating with a wild male is revealed by sperm impregnation of the spermathecae of the recaptured female and for *palpalis* species by the presence of mating scars. First laboratory observations with gamma treated *G. palpalis palpalis* females, revealed a normal receptivity to mating and insemination rate (Van der Vloedt & Barnor, 1984). On the island of Unguja (Zanzibar), *Glossina austeni* is the only tsetse species present and solely responsible for the cyclical transmission of trypanosomiasis (Johns, 1951). Control operations, undertaken by the Tsetse Unit of the Department of Livestock Development are assisted by the UNDP/FAO Animal Disease project and IAEA Technical Co-operation project "The eradication of *G. austeni* from Unguja by means of the Sterile Insect Technique". Monitoring operations however, are hampered by the elusiveness of the fly and the low apparent densities over large parts of the island. The use of virgin gamma sterilised female *G. austeni* is anticipated to increase the efficiency of the global monitoring activities on the island.

Therefore, laboratory studies were initiated to examine:

1. the dynamics of follicle development in gamma treated females
2. receptivity, insemination status and fecundity of females in relation to timing of treatment and female age at mating,
3. the rate of multiple mating of untreated and treated females, and
4. the mating preference of untreated males in the presence of equal densities of treated and untreated females at different ages.

The results of the study are presented in this paper.

MATERIAL AND METHODS

Experimental flies

The flies used in the experiments originated from Unguja (Zanzibar) island, United Republic of Tanzania, and are maintained at the Entomology Unit of the IAEA's Laboratories in Seibersdorf (Austria) on a membrane feeding system since 1986. The flies were kept under constant climatic conditions of 24° ± 1°C and at Relative Humidity of 85% ± 5% and fed 6 times a week through silicone rubber membrane on frozen and thawed bovine blood.

Experimental procedures

1. FOLLICLE DEVELOPMENT IN GAMMA TREATED FEMALES

Virgin females were given a treatment (60 Gy in air) in a ^{60}Co source on day 2 or day 9 following emergence and a batch of pupae was treated (50 Gy in air) on day 33 following larviposition. Treated females were mated when 2 days old with untreated sexually mature colony males. After separation, females were kept in standard colony cages and groups of at least 5 females were dissected every 5 days for examination of the insemination status and ovarian configuration. Follicle length was measured with an ocular micrometer in the eyepiece of a phase-contrast Leitz compound microscope at x 64 magnification.

2. MATING RESPONSE AND FECUNDITY OF UNTREATED AND IRRADIATED FEMALES

Virgin females and a batch of pupae were exposed to gamma radiation in air at doses of 60 Gy on day 2 or 9 following emergence and 50 Gy on day 33 post larviposition respectively. Receptivity of 3 to 15 day old treated (n = 40) and untreated females (n = 40) was assessed by exposing them (20 females per cage) to the same number of sexually mature males in a cage with a diameter of 20 cm and 4.5 cm high. Immediate mating response was expressed as the number of females observed *in copula* within the first 30 minutes of confinement. Mating pairs were isolated in plastic tubes (2.5 cm diameter and 6 cm high) and copulation time recorded. After separation, females were pooled in

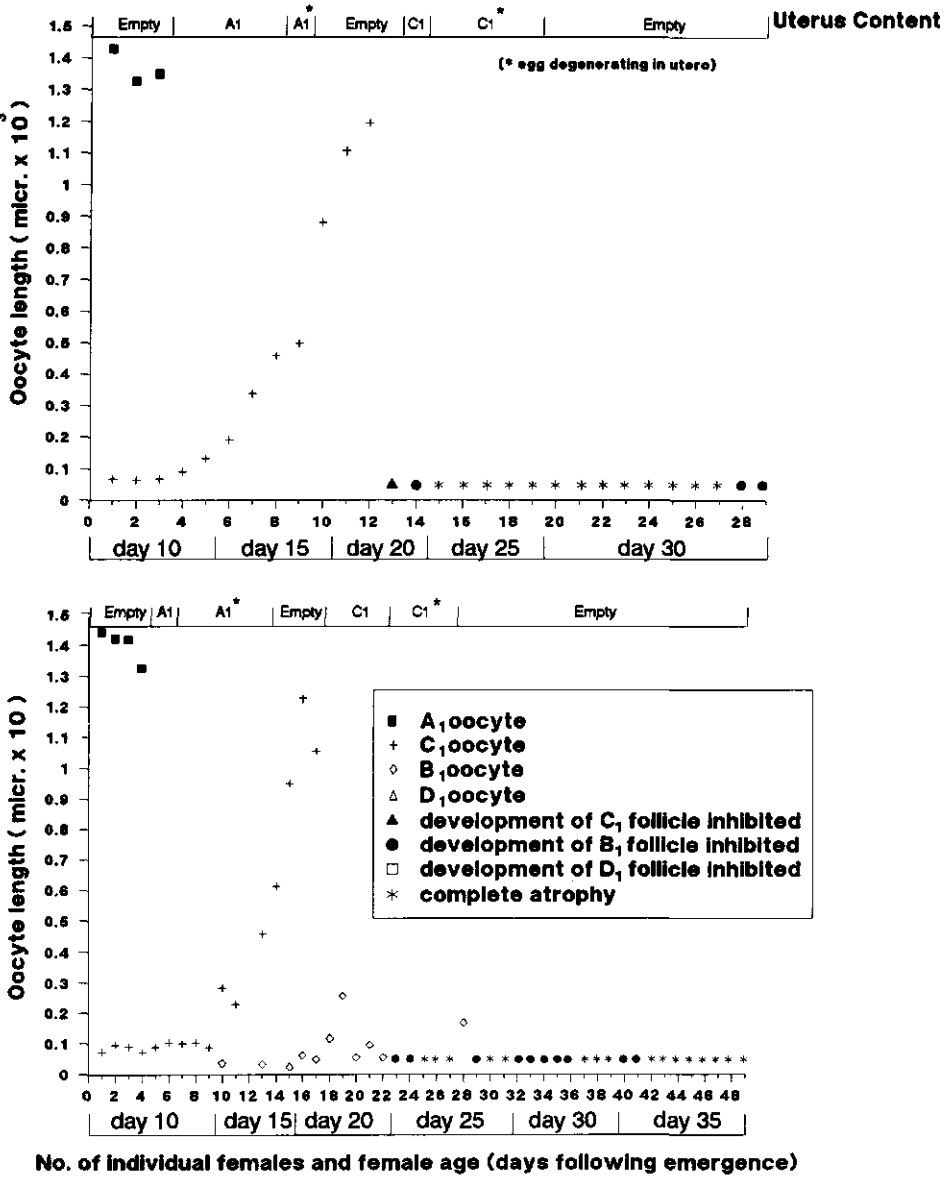

Fig. 1 Follicle development and uterus content of *G. austeni* females, irradiated with 50 Gy on day 33 post larviposition (top graph) and with 60 Gy on day 2 following emergence (bottom graph). Females were mated with untreated males.

standard colony cages and survival and pupae production monitored for 45 days. Larviposition receptacles were examined for aborted eggs and immature larvae every 5 days. At the end of the experimental period, all females were dissected and their reproductive system examined microscopically.

3. MULTIPLE MATING BEHAVIOUR

Virgin females were given an irradiation treatment (60 and 120 Gy in air) on day 2 (group I) and day 5 (group II) post emergence. A first mating opportunity was offered on day 3 (group I) and day 5 (group II) and receptivity assessed as described above. After termination of the mating act however, males were immediately removed, females left singly in the plastic tubes and on day 4,7,9 and 15 for the first group and on day 6,8,11 and 14 for the second group, the females were offered a 2nd, 3th, 4th and 5th mating opportunity.

4. MATING PREFERENCE AND RECEPTIVITY TESTS

The mating preference of untreated males and the receptivity to mating of females with increasing age was assessed by exposing the males to equal numbers of treated and untreated females. Each of 5 containers (32 cm high, 20 cm diameter) was filled with differently colour-marked untreated (UT)(N=40) and 60 Gy treated (T)(N=40) females of the following ages : + 2 days, + 5 days, + 8 days, + 11 days and + 14 days. For each container, 4 x 10 males were introduced, the mating pairs were removed and the type of female determined.

RESULTS

Follicle development of irradiated females

Follicle development of females treated as pupae with 50 Gy on day 33 following larviposition and with 60 Gy on day 2 and 9 following emergence is illustrated in Fig. 1 and 2. In all females, the radiation treatment did not inhibit vitellogenesis supported by the nurse cells of the A_1 (first follicle in ovulation sequence A.C.B.D., internal right) and C_1 follicle (second follicle to ovulate, internal left) resulting in normal growth and maturation. Only in one 20 day-old female (treated in pupal stage) was maturation of the C_1 follicle interrupted. Mature oocytes in position A_1 and C_1 ovulated normally in all flies, but no development

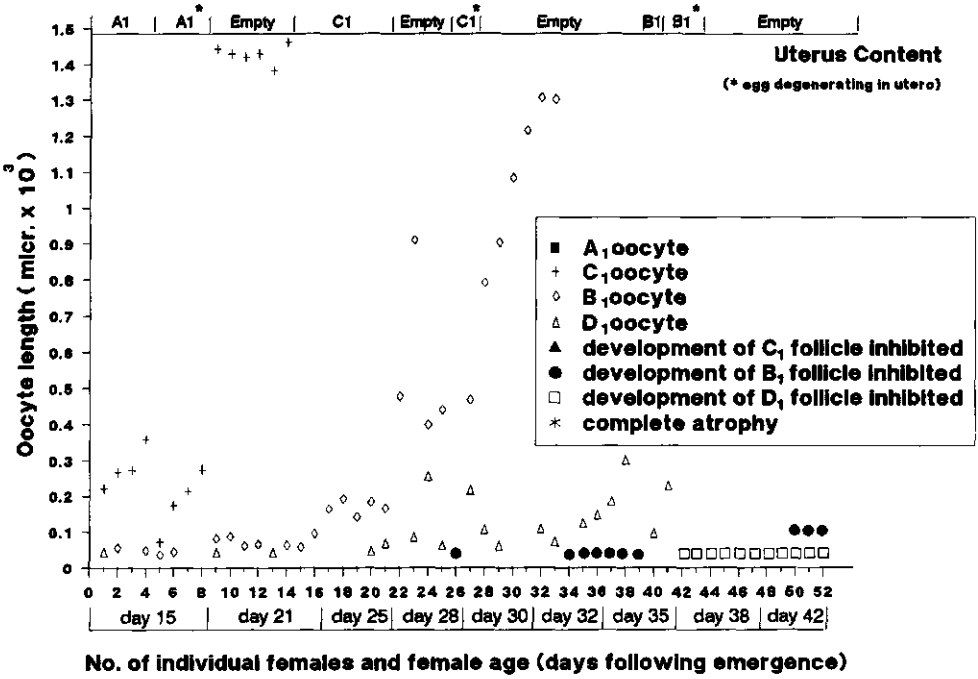

Fig. 2 Follicle development and uterus content of *G. austeni* females, irradiated with 60 Gy on day 9 following emergence. Females were mated with untreated males.

occurred *in utero* beyond the egg stage. All degenerating A_1 and C_1 oocytes were extruded or absorbed.

No differentiation between oocyte and nurse cells was observed for the B_1 and D_1 follicles (third and fourth in ovulation sequence respectively) in 89.6% of the females treated as pupae (Fig. 1, top). Evidence for an initial development of a B_1 follicle was found in 3 females (10.3%), but development was inhibited before reaching the maturation stage. From day 15 on, all flies displayed inactive ovaries characterised by atrophied oocytes and nurse cells.

In 85% of 15-21 day old females, treated on day 2 following emergence (Fig. 1, bottom), the oocyte in position B_1 could be differentiated from its nurse cells but no advanced stage of maturation of the B_1 follicle was found. Consequently, no flies were observed with

Table 1. Receptivity and fecundity of irradiated *G. austeni* females, treated as pupae and at various ages following emergence. Adult females were treated with 60 Gy, pupae with 50 Gy

Treatment	Mating	Mating Response % [1]	Duration of copulation (min.) < 60 %	60 -< 120 %	120 -< 180 %	> 180 %	Insemination rate %	Survival day 45 % [2]	Fecundity [3]	Extruded eggs recovered No./female
Control	day 3	91.1	0.0	7.3	46.3	46.3	100	99.0	0.084	0.02
Control	day 5	86.7	7.7	5.1	59.0	28.2	91.4	99.7	0.085	0.17
Control	day 7	77.8	5.7	34.3	51.4	8.6	97.0	97.1	0.076	0.24
Control	day 12	82.2	2.7	56.8	37.8	2.7	97.1	96.3	0.068	0.11
Control	day 15	77.8	22.9	68.6	8.6	0.0	93.8	93.2	0.052	0.35
day 33 PL *	day 3	84.4	2.6	7.9	34.2	55.3	97.3	92.8	0.000	0.97
day 33 PL *	day 9	64.7	20.0	20.0	50.0	10.0		no record		
day 33 PL *	day 14	85.0	5.9	52.9	41.2	0.0		no record		
day 2	day 3	86.7	0.0	7.9	39.5	52.6	97.3	94.9	0.000	0.95
day 2	day 7	77.8	14.7	26.5	52.9	5.9	96.9	97.0	0.000	1.09
day 2	day 9	68.9	10.0	66.7	23.3	0.0	96.4	99.5	0.000	1.46
day 2	day 15	84.4	23.7	63.2	10.5	2.6	95.0	100	0.000	1.28
day 9	day 10	80.0	5.6	30.6	44.4	19.4	100	97.3	0.000	1.73

1. Observed pairs during the first 30 min. of confinement
2. Survival relative to mature female days
3. No. pupae/mature female day

* Irradiated as pupae

an ovulated B_1 *in utero.* Maturation of the B_1 follicle was inhibited in 38.5% of 25-35 day old females, whereas, complete atrophy was observed in 61.5% of the females. No evidence of development was found for a follicle in position D_1 (4 th in ovulation sequence, external left).

A normal development of the B_1 follicle was observed in 55% of 28 - 35 day old females treated on day 9 following emergence (Fig. 2). However, in 35% of the dissected females, the maturation process was inhibited. Young differentiated D_1 follicles were observed in 40% of the dissected females from day 21 on, but none fully matured.

Receptivity and fecundity of untreated and irradiated females

Data on receptivity and performance of treated and untreated females are presented in Table 1. Untreated females displayed the highest receptivity on day 3 and 5 following emergence, with 91% and 86% of the females observed *in copula* within 30 minutes. Mating of untreated females at older ages (7 - 15 days old) resulted in a mating response of at least 77%. No major differences in receptivity were observed for treated females as compared to control females, with neither the moment of treatment nor the age at mating having any influence. The duration of copulation decreased in accordance with increasing age at mating i.e. 92% of untreated females, mated on day 3, copulated longer than 120 minutes, whereas 59.5% and 91.5% of untreated females mated on day 12 and day 15 respectively were observed *in copula* for less than 120 minutes. The same trend was found with irradiated females. The radiation treatment administered to pupae 3 days before emergence and to adult females had no deleterious effect on their survival (> 92% by day 45 for all treatment groups) and insemination rate (> 95%). Fecundity of the untreated control females decreased from 0.084 pupae/mature female day for females mated on day 3 to 0.068 and 0.052 pupae/mature female day for females mated on day 12 and 15 respectively. In addition, the number of extruded eggs/initial female increased from 0.02 (females mated on day 3) to 0.35 (females mated on day 15). No larvae were produced by the females of the different treatment groups and the number of recovered dead eggs varied from 0.9 to 1.7 per initial female.

Dissection of the females on day 45 revealed marked differences in the reproductive status of untreated and treated females. As expected, untreated females always displayed an egg undergoing normal embryogenesis or a developing larva *in utero* whereas treated females were always found with a degenerating egg *in utero* or an uterus empty

Table 2. Receptivity of untreated (UT) and treated (T)(60 Gy in air) *G.austeni* females in equal population densities (2 x 40) and mating preference of untreated *G.austeni* males

	FEMALE AGE (Days following emergence)									
	+ 2 days		+ 5 days		+ 8 days		+ 11 days		+ 14 days	
No. of males introduced	Type of female found *in copula* UT	T	Type of female found *in copula* UT	T	Type of female found *in copula* UT	T	Type of female found *in copula* UT	T	Type of female found *in copula* UT	T
10	4	6	4	6	5	5	4	6	5	5
10	4	6	5	5	2	8	5	5	5	5
10	4	6	5	5	6	4	7	3	6	4
10	5	5	5	5	3	7	6	4	4	6
Total	17	23	19	21	16	24	22	18	20	20

due to recent expulsion of the egg. Dissection results of the treated females confirmed the above made observations on follicle dynamics, i.e. 97.1%, 18.9% and 16.7% of the females treated as pupae, as adults on day 2 and 9 after emergence respectively, were found with completely inactivated ovaries on day 45 (oocyte degeneration and nurse cells atrophied). Inhibition of the maturation process of the follicle in position B_1 and D_1 was found in 2.9%, 81.1% and 83.3% of the females treated as pupae, on day 2 and 9 following emergence respectively.

Multiple mating rate

A high rate of multiple mating was observed when untreated and females treated with 60 Gy on day 2 were offered several mating opportunities (Fig. 2). 74.2%, 52.0% and 24.0% of untreated females and 77.1%, 38.9% and 23.8% of the treated females accepted a male on a second, third and fourth mating occasion respectively. None of the untreated females ($n = 6$) accepted a male on day 15 (5 th mating occasion) but 2 treated females ($n = 5$) were willing to copulate for the fifth time. A higher radiation dose administered on day 2, gave a mating response of 83.8% on a first mating occasion (day 3), but receptivity dropped to 56.1%, 29.7% and 9.1% on later mating opportunities. Receptivity to remating was decreased when treatment was given on day 5 following emergence.

An average mating time of 208 ± 54 min., 199 ± 58 min. and 200 ± 58 min. was recorded for the first mating of untreated, 60 Gy and 120 Gy treated females respectively. Although variations occurred in average mating time for later matings, both untreated and treated females tended to mate less long with increasing age and number of matings (Fig. 3).

Mating preference and receptivity

Table 2 indicates that untreated males, irrespective of the age of the females at mating day, had no obvious preference for untreated or treated females. Although treated females were more receptive to mating in most of the test series, no significant deviations from the 1:1 (UT:T) ratio were found ($p > 0.05$).

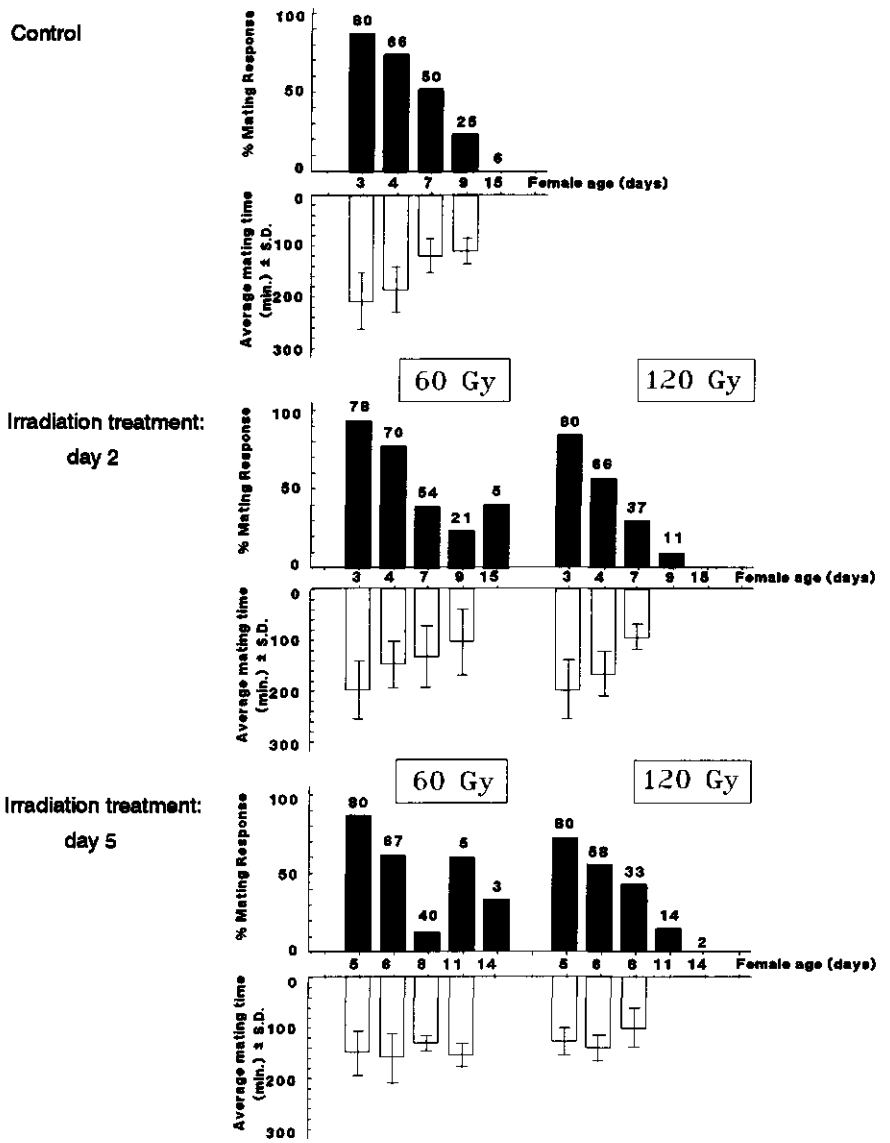

Fig. 3. Mating response and average mating time of untreated and irradiated *G. austeni* females, mated with untreated males, during 5 mating opportunities (on day 3,4,7,9 and 15 for control and treatment group day 2; on day 5,6,8,11 and 14 for treatment group day 5). (Figures on top of bars indicate number of experimental females).

DISCUSSION

The findings of Van der Vloedt & Barnor (1984) that relatively low doses (50 - 60 Gy) of gamma radiation result in complete sterility in female *Glossina palpalis palpalis*, without altering their mating behaviour are corroborated by our study with female *G. austeni*. In addition, during laboratory cage tests with equal numbers of untreated and treated females, untreated males showed no significant preference to mating with either treated or untreated females.

Moreover, the present study revealed that the age of the female when the radiation treatment is administered influences significantly the dynamics of the follicle development. Saunders (1960) has shown that the ovarian development in untreated tsetse females is characterised by a sequential maturation of the 4 egg follicles. Although ovaries are already present in the pupae seven days after larviposition (Riordan, 1970), differentiation of oogonia in oocyte and nurse cells in the internal ovarioles and subsequently the onset of vitellogenesis supported by the nurse cells is initiated 5 - 10 days before emergence (Van der Vloedt *et al.*, 1976). In general, radiosensitivity of cells can be related to their proliferative activity and mitotic figure, and inversely to their degree of differentiation (Ducoff, 1972). The most radiosensitive stage encountered in *G. p. palpalis* is when the nurse cells undergo endomitotic replication of chromosomal material (Van der Vloedt & Barnor, 1984). From our observations on follicle dynamics in female *G. austeni*, endomitosis in follicles A_1 and C_1 is apparently already completed at the moment of irradiation, even when the treatment is given on day 33 post larviposition. In addition, nurse cell function was not adversely affected by the radiation treatment as vitellogenesis could proceed to completion resulting in fully matured follicles in position A_1 and C_1.

In females treated as pupae, the absence of any differentiation between oocyte and nurse cells of follicles in position B_1 and D_1 may be associated with the lethal treatment given to the germinal tissue. No development is observed beyond the maturation of follicles A_1 and C_1, and the ovaries enter the phase of inactivity with females displaying completely atrophied ovaries. Treatment given to adult females, resulted however in different degrees of development of the follicles in position B_1 and D_1, depending on the timing of the treatment. The failure to form mature ova is more pronounced when treatment is given in early life. Treating 2-day old females apparently coincides with nurse cell endomitosis of follicle B_1, and consequently,

normal nurse cell function is hampered resulting in cessation of the vitellogenesis process. In addition, the fact that follicles in position B_1 did not reach maturity in 35% of the females treated on day 9, might be an indication of radiation induced damage to the oocyte nucleus and its adverse effect on normal nurse cell function (LaChance & Bruns, 1963).

The willingness of female *G. austeni* to accept more than once a mating in captivity was demonstrated by Curtis (1968). The same observation was made for *Glossina palpalis palpalis* by Jordan (1958) and Van der Vloedt *et al.* (1978). The present study not only revealed that irradiated female *G. austeni* exhibit an extensive multiple mating behaviour, but also that higher radiation doses (120 Gy) and treating flies later in life (day 5) tend to decrease their remating ability.

Our findings on receptivity, mating behaviour and mating preference strongly indicate the feasibility of using radiosterilised *G. austeni* females in the field in release-recapture exercises. After completion of control operations, it is of uttermost importance to have the assurance that no residual fly populations are present which can re-infest cleared areas. The method however, will be limited to a yes/no determination whether relic fly pockets exist or not. Our observations on the extensive multiple mating capacity of irradiated females in captivity and the absence of mating scars (which can expose multiple matings in *palpalis* species) indicate that quantitative assessments of population densities might not be feasible. Nevertheless, this high rate of re-mating of irradiated females could be exploited in operational eradication campaigns, by releasing large quantities of sterile females in an area where prior suppression of the wild fly population has been achieved, and where releases of sterile males have not yet been initiated. The high frequency of mating of the released females might cause severe sperm depletion in wild males.

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Chapter 7

THE EFFECT OF IONISING RADIATION ON THE REPRODUCTIVE BIOLOGY OF *Glossina tachinoides* Westwood FEMALES: A COMPARATIVE STUDY

Abstract

The receptivity to mating, insemination status and productivity of untreated virgin *Glossina tachinoides* was compared with virgin females treated with a gamma radiation dose of 40 Gy between day 2 and 6 following emergence. Mating response and insemination were not negatively affected by the radiation treatment. On the contrary, irradiated females remained significantly more receptive to mating at older ages. No offspring was produced by the irradiated females.

In all treated females, the follicles in position A_1 (internal right) and C_1 (internal left) developed normally to maturity. After ovulation, the dead eggs were however expelled and the flies displayed completely atrophied ovaries. Females treated later in adult life, displayed different degrees of development of the follicles in position B_1 (external right) and D_1 (external left). Untreated male flies, exposed to equal densities of untreated and treated female flies, showed no mating preference for either untreated or irradiated 2 - 12 day old females.

Treated females were more receptive to multiple matings as compared to untreated females. The multiple mating rate declined with increased radiation doses and when treatment was administered later in the female life.

Table 1. Receptivity and fecundity of *G. tachinoides* females irradiated with 40 Gy at various ages following emergence (45 females per test series)

Treatment (40 Gy)	Mating	Mating Response % [1]	Duration of copulation (min.)					Insemination rate %	Survival day 45 % [2]	Fecundity [3]	Extruded eggs recovered No./female
			< 30 %	30 -< 60 %	60 -< 90 %	90 -< 120 %	> 120 %				
Control	day 3	88.9	0.0	40.0	20.0	22.5	17.5	100	96.2	0.089	0.26
Control	day 7	68.9	0.0	74.2	12.9	6.5	6.5	100	98.8	0.087	0.24
Control	day 9	26.7	18.2	45.5	18.2	0.0	18.2	100	61.3	0.067	0.31
Control	day 15	22.2	30.0	10.0	60.0	0.0	0.0	88.8	91.1	0.047	0.33
day 2	day 3	82.2	0.0	21.6	51.4	8.1	18.9	94.6	90.6	0.000	1.62
day 2	day 7	93.3	0.0	31.0	45.2	11.9	11.9	100	86.3	0.000	1.67
day 2	day 9	88.9	2.5	40.0	22.5	22.5	12.5	87.2	93.4	0.000	1.66
day 2	day 15	33.3	13.3	73.3	13.3	0.0	0.0	78.6	93.3	0.000	1.20
day 4	day 5	91.1	0.0	29.3	29.3	19.5	22.0	100	97.7	0.000	1.93
day 4	day 7	93.3	2.4	45.2	26.2	19.0	7.1	95.0	93.6	0.000	1.76
day 4	day 9	86.7	2.6	61.5	23.1	7.7	5.1	88.9	90.8	0.000	1.56
day 4	day 12	62.2	32.1	67.9	0.0	0.0	0.0	76.0	88.0	0.000	1.61
day 6	day 7	84.4	5.3	23.7	23.7	34.2	13.2	97.3	98.7	0.000	1.89
day 6	day 12	51.1	17.4	60.9	17.4	4.3	0.0	90.9	89.0	0.000	1.52
day 6	day 15	42.2	10.5	52.6	26.3	10.5	0.0	N.D.	93.0	0.000	1.47

1. Observed pairs during the first 30 min. of confinement
2. Survival relative to mature female days
3. Pupae/mature female day

INTRODUCTION

The dynamics of wild tsetse populations can be monitored by standard trapping techniques as long as the fly densities are sufficiently high for detection (Challier, 1982). During and after control, fly densities can however be below the detectable level of the used trapping device. The presence of remaining flies or pockets of isolated relic flies can then be exposed by the release-recapture of gamma sterilised female tsetse flies (Van der Vloedt & Barnor, 1984). Laboratory studies have already shown that female *Glossina palpalis palpalis* (Van der Vloedt & Barnor, 1984) and female *Glossina austeni* (chapter 6) were completely sterilised after exposure to low doses of gamma radiation without any significant alteration in their mating behaviour and insemination status.

No data are at present available on the effect of gamma radiation on female *Glossina tachinoides*. Therefore, a study was carried out to examine the following aspects related to the females' reproductive biology: (1) receptivity, insemination status and productivity of untreated and irradiated females at various ages following emergence, (2) the rate of multiple mating of irradiated females as compared to untreated females and (3) the mating preference of untreated males in the presence of equal densities of treated and untreated females. The results are discussed and compared with data obtained for other tsetse species.

MATERIAL AND METHODS

All *Glossina tachinoides* females used in the experiments were derived from the stock colony maintained at the Entomology Unit of the IAEA's Laboratory in Seibersdorf, Austria. The colony was initiated with flies originating from Burkina Faso. All experimental flies were kept in the insectary together with the mother colony under standard holding conditions i.e. 23° ± 1°C. and R.H. of 75% ± 5% (Bauer *et al.*, 1984; Van der Vloedt *et al.*, 1987). Flies were fed 6 times a week on fresh frozen and thawed bovine and porcine blood (Wetzel & Luger, 1978).

Except for some minor alterations, all experimental procedures to assess mating response and fecundity of untreated and irradiated females, the multiple mating behaviour and mating preference of untreated males in equal population densities of untreated and treated females were in essence identical to the ones described in a previous paper (chapter 6). In the receptivity tests, a dose rate of 40 Gy was applied in air to virgin adult females on day 2, 4 and 6 following

Table 2. Reproductive status of treated (40 Gy in air) and untreated *G. tachinoides* females inseminated by untreated colony males

Treatment	Mating	Ovarian configuration (%)								Uterus Content (%)					
		A_2	C_2	B_1	D_1	Blockage	complete atrophy	left follicle lysed	right follicle lysed	Uterus empty	Recently ovulated egg	Degenerating egg	Viable instar larva		
													L1	L2	L3
Control	day 3	85.7	8.6	0.0	2.9	2.9	0.0	0.0	0.0	8.6	17.1	0.0	5.7	45.7	22.9
Control	day 7	84.6	0.0	3.8	0.0	11.5	0.0	0.0	0.0	6.9	13.8	3.4	0.0	51.7	24.1
Control	day 9	85.7	14.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	57.1	0.0	14.3	14.3	14.3
Control	day 15	85.7	0.0	0.0	0.0	14.3	0.0	0.0	0.0	0.0	0.0	0.0	14.3	42.9	42.9
day 2	day 3	0.0	0.0	0.0	0.0	0.0	97.0	0.0	3.0	100	0.0	0.0	0.0	0.0	0.0
day 2	day 5	0.0	0.0	0.0	0.0	0.0	97.1	0.0	2.9	100	0.0	0.0	0.0	0.0	0.0
day 2	day 7	0.0	0.0	0.0	0.0	0.0	93.9	0.0	6.1	100	0.0	0.0	0.0	0.0	0.0
day 2	day 15	0.0	0.0	0.0	0.0	0.0	90.9	0.0	9.1	100	0.0	0.0	0.0	0.0	0.0
day 4	day 5	0.0	0.0	0.0	0.0	0.0	92.9	0.0	7.3	100	0.0	0.0	0.0	0.0	0.0
day 4	day 7	0.0	0.0	0.0	0.0	0.0	90.9	6.1	6.1	100	0.0	0.0	0.0	0.0	0.0
day 4	day 9	0.0	0.0	0.0	0.0	0.0	100	0.0	0.0	100	0.0	0.0	0.0	0.0	0.0
day 4	day 12	0.0	0.0	0.0	0.0	0.0	88.2	11.8	11.8	100	0.0	0.0	0.0	0.0	0.0
day 6	day 7	0.0	0.0	0.0	0.0	0.0	94.4	0.0	5.6	100	0.0	0.0	0.0	0.0	0.0
day 6	day 12	0.0	0.0	0.0	0.0	0.0	81.3	0.0	18.8	100	0.0	0.0	0.0	0.0	0.0
day 6	day 15	0.0	0.0	0.0	0.0	0.0	100	0.0	0.0	100	0.0	0.0	0.0	0.0	0.0

emergence. Females were mated with sexually mature untreated colony males on day 3,5,7 or 15 post emergence (irradiation on day 2), on day 5,7,9 or 12 post emergence (irradiation on day 4) and on day 7,12 and 15 for females irradiated on day 6. During mating preference tests, untreated sexually mature male flies (n = 40) were introduced in groups of 10 to untreated and treated females kept in equal densities in a container of following dimensions: 32 cm high and 20 cm diameter. The experiment was carried out with females of the following age: +3 days, + 5 days, + 7 days, +9 days and +12 days. For the multiple mating tests, females were irradiated with 40 Gy or 120 Gy on day 2 or 5 after emergence and given a mating opportunity on day 3, 4, 7, 9 and 15 for the first group and on day 5, 6, 8 and 11 for the second irradiation group.

RESULTS

Receptivity and fecundity of untreated and irradiated females

Untreated virgin females, mated on day 3 following emergence showed a high immediate mating response (88.9% of the females were observed *in copula* during the first 30 minutes of confinement), 100 % insemination rate resulting in a fecundity of 0.089 pupae per mature female day (Table 1). The immediate response to mating declined to 68.9%, 26.7% and 22.2% when a first mating opportunity was offered on day 7, day 9 and day 15 respectively. Although insemination rate of all untreated females remained above 88.8%, fecundity was proportionally reduced when mated later in life. Marked changes were observed in the mating behaviour of untreated females related to their age at mating i.e. whereas 40% of the virgin females mated on day 3 post emergence (PE) required more than 90 minutes to complete the mating act, none of the females mated on day 15 were observed *in copula* for longer than 90 minutes. On the contrary, 40 % of the females mated less than 60 minutes.

The immediate mating response of the irradiated virgin females was always significantly higher (chi square, $p < 0.01$) as compared to untreated females irrespective of the treatment day or the mating day (except for females irradiated on day 2 and mated on day 15, $p > 0.05$) i.e. between 84% and 93% of the irradiated females accepted a male on a first mating opportunity on day 7 (versus 68.9% for untreated females). Even when mated on day 15 after emergence, between 33.3% and 42.2% of the irradiated females were receptive to mating. At least 76% of all irradiated females were inseminated but no viable offspring

Table 3. Receptivity of untreated (UT) and treated (T)(40 Gy in air) *G.tachinoides* females in equal population densities (2 X 40) and mating preference of untreated *G.tachinoides* males

	FEMALE AGE (Days following emergence)										
	+ 3 days		+ 5 days		+ 7 days		+ 9 days		+ 12 days		
No. of males introduced	Type of female found *in copula* UT	T	Type of female found *in copula* UT	T	Type of female found *in copula* UT	T	Type of female found *in copula* UT	T	Type of female found *in copula* UT	T	
10	3	7	5	5	5	5	5	4	1	6	*
10	4	6	5	5	4	6	4	7	1	3	**
10	7	3	5	5	4	6	4	4	1	3	***
10	5	5	6	4	5	2	2	8	3	1	****
Total	19	21	21	19	18	22	17	23	6	13	

* 3 males failed to copulate
** 6 males failed to copulate
*** 6 males failed to copulate
**** 6 males failed to copulate

was produced. On the contrary, between 1.2 and 1.9 dead embryos were recovered per initial female during the experimental period of 45 days. As observed with untreated females, mating time declined with increasing mating age.

The reproductive status (ovarian configuration and uterus content) of irradiated females as displayed by dissection on day 45 is presented in Table 2 and compared with untreated females. In untreated females, the sequential development of the ovarioles proceeded normally with 85% of the dissected females showing the A_2 follicle (internal left) as the next in ovulation sequence A.C.B.D. on day 45. Ovulation was however inhibited leading to egg retention in the ovaries (i.e. blocked ovaries) in 11 - 14% of the females who had mated on day 7 or later. The uterus of all untreated females contained a recently ovulated egg undergoing normal embryogenesis or a developing larva. In 8.6% of the untreated females, the uterus was empty due to recent deposition of a larva.

Table 2 shows that a complete aberrant reproductive picture was revealed when irradiated females were dissected i.e. in all irradiated females, the uterus was empty and in 81 to 100% of the females, the ovarioles were completely atrophied. The proportion of flies with a left or right follicle "lysed" (i.e. evidence of onset of maturation of the oocyte, but maturation process inhibited) increased from 3-9% to 6 - 19% for the females treated on day 2 and day 6.

Mating preference and receptivity tests

Table 3 presents the results of a mating test where untreated, sexually mature colony male flies (4 x 10) were introduced in a population of treated and untreated females (aged between 3 and 12 days) of equal density (2 x 40). Untreated males had no obvious preference for mating with either untreated or irradiated females aged between 3 and 9 days, and both types of females seemed equally receptive to mating ($p > 0.05$). When males were introduced in a population of 12 day old females, 21 males out of 40 failed to find a female mate for copulation. Of the 19 males who did mate, 13 (68.4%) did so with irradiated females ($p > 0.05$).

Multiple mating

Table 4 presents the immediate mating response and average mating time of untreated and irradiated females (with 40 Gy or 120 Gy on day 2 or 5 PE) offered multiple mating opportunities. 67% of the untreated

Table 4. Multiple mating behaviour of irradiated and untreated *G. tachinoides* females

Irradiation day/dose	Mating	No. of females	Mating Response % [1]	Average mating time (min.) ± SD
Control	day 3	80	76.3	78 ± 45
	day 4 (2 nd)	61	67.2	96 ± 26
	day 7 (3 rd)	42	26.2	53 ± 17
	day 9 (4 th)	9	11.1	no record
	day 15 (5 th)	1	0.0	no record
day 2 / 60 Gy	day 3	80	81.3	76 ± 29
	day 4 (2 nd)	65	80.0	94 ± 35
	day 7 (3 rd)	52	42.3	55 ± 22
	day 9 (4 th)	22	27.3	42 ± 10
	day 15 (5 th)	6	0.0	no record
day 2 / 120 Gy	day 3	80	86.3	91 ± 35
	day 4 (2 nd)	68	67.6	89 ± 34
	day 7 (3 rd)	45	28.9	43 ± 13
	day 9 (4 th)	12	16.7	40 ± 7
	day 15 (5 th)	2	1 / 2	no record
day 5 / 60 Gy	day 5	80	67.5	64 ± 20
	day 6 (2 nd)	53	47.2	59 ± 21
	day 8 (3 rd)	25	48.0	48 ± 8
	day 11 (4 th)	11	0.0	no record
day 5 / 120 Gy	day 5	80	71.3	46 ± 14
	day 6 (2 nd)	57	22.8	55 ± 17
	day 8 (3 rd)	13	53.8	44 ± 13
	day 11 (4 th)	7	1 / 7	no record

1. Observed pairs during the first 30 min. of confinement

females who had already mated once on day 3 PE, were receptive to a second mating on day 4 PE. Only 26% and 11.1% (1 female out of 9) of these untreated females accepted a male mate on a third (day 7) and fourth mating (day 9) occasion respectively.

Females, irradiated with 40 Gy on the second day after emergence, were significantly (chi square, $p < 0.01$) more receptive to multiple matings as compared to untreated females (80%, 42% and 27% of the females accepted a male on a second, third and fourth mating occasion). When the radiation treatment was increased to 120 Gy, the multiple mating rate was reduced and comparable to the untreated females (chi square, $p > 0.05$) (67%, 29% and 16% mating pairs observed on a second, third and fourth mating opportunity respectively). Likewise, the multiple mating rate declined when females were treated with 40 Gy on day 5 of their adult life but increased again with 120 Gy (significant for 2nd and 3 rd mating, $p < 0.01$).

DISCUSSION

During their radiation studies with *Glossina morsitans* and *Glossina pallidipes*, Dean & Wortham (1969) and Dean & Clements (1969) demonstrated the higher radiosensitivity of female tsetse flies as compared to males. Female production was completely inhibited after exposure to a radiation dose as low as 40 Gy. These findings were later confirmed by more detailed analysis of species belonging to the *palpalis* group. Exposing 25-27 day old female *G. p. palpalis* pupae to 10.5 Gy of fast neutrons (Van der Vloedt *et al.*, 1976), treating adult female *G. p. palpalis* with 60 Gy of gamma radiation (Van der Vloedt & Barnor, 1984) and exposing 25 - 28 day old female *G. tachinoides* pupae to a dose of 40 Gy in a ^{60}Co source (chapter 3) resulted in a complete loss of female fecundity. The same observation was made for *G. austeni*, a member of the *morsitans* group. A treatment of 50 Gy during the late pupal phase and 60 Gy in the adult stage induced complete sterility (chapter 6).

In all tsetse species studied up to now, including the data on *G. tachinoides* presented in this study, female receptivity to mating was not negatively affected by the radiation treatment. On the contrary, gamma irradiated female *G. tachinoides* remained significantly more receptive to mating at older ages as compared to untreated females. This was confirmed by laboratory cage tests with untreated males in equal population densities of untreated and irradiated females. These observations are different from the data available for gamma treated *G.*

p. palpalis (Van der Vloedt & Barnor, 1984) and *G. austeni* (chapter 6). During the various studies on mating behaviour of different tsetse species, distinct interspecific differences have become apparent. With the exception of *G. pallidipes* (Rogers, 1972), virgin female tsetse flies are in general most receptive to accept a male, with maximal insemination rates, during the first 2-3 days after emergence (Jordan, 1958; Saunders, 1970; Nash *et al.*, 1971). Receptivity to mating declines normally with increasing female age (Jordan, 1958; chapter 6) and most female flies are reluctant to mate after the first ovulation. Extended receptivity has been attributed to a delay in follicle development (Van der Vloedt & Barnor, 1984). Whereas untreated virgin *G. austeni* females remained highly receptive to mating up to day 15 following emergence (77.8%) (chapter 6), the immediate mating response of 15 day old virgin female *G. p. palpalis* and *G. tachinoides* was reduced to 56% (Van der Vloedt & Barnor, 1984) and 22.2% respectively (this study). In addition, the time required to complete a normal copulation was likewise species specific i.e. the majority of 2-day old virgin *G. p. palpalis* and *G. austeni* females mated longer than 90 and 180 minutes respectively, whereas more than 60% of the female *G. tachinoides* of the same age had already completed the mating act after 60-90 minutes. In all three species however, the mating time of virgin untreated females was reduced when mated later in the adult life. The same observation was made when females had received a gamma radiation treatment. The fact that a sexually mature male fly, who has not mated before, requires less time to transfer a spermatophore if mated with an older female, is indicative for the significant contribution made by the female tsetse fly in the control of the mating act. The factors which determine the length of a normal copulation and the observed differences between the species are not clear. The apparent inter-specific differences in female receptivity to mating are most likely related to differences in timing of the first ovulation which is possibly influenced by the prevailing climatic conditions (temperature) and the amount of embryonic reserves in the emerging fly.

The timing when the irradiation treatment is administered influenced significantly the dynamics of the follicle development in *G. austeni* females (Vreysen & Van der Vloedt, 1992). Treating females during the late pupal phase resulted in normal maturation of the follicles in A_1 and C_1 position (Saunders, 1960), normal ovulation and expulsion of the egg. Treatments in the adult female life, resulted in addition to a partial maturation of the B_1 and/or D_1 follicle but follicle growth was inhibited before reaching full maturity. The dissection

results of the *G. tachinoides* females at the end of the tests combined with the observed amount of eggs expelled during the experimental period, substantiated these observations. No interruption occurred for the first two reproductive cycles (follicles A_1 and C_1), however without any production of offspring. Treatments later in adult life resulted in partial development of the follicles in B_1 and D_1 position.

A prerequisite for the successful completion of a tsetse control programme is the confirmation that no residual pockets of flies exist in the "cleared" area. Several methods are at the disposal of tsetse control officers to consolidate the status of eradication: (a) sentinel animal herds can be introduced in strategic localities and the screening of their blood, sampled at regular intervals, gives an indication of potential transmission i.e. presence of tsetse flies. Although very sensitive screening techniques (e.g. Ag-ELISA) (Nantulya & Lindqvist,1989) are currently available to expose trypanosome infections in livestock, a sentinel herd scheme is very time consuming, expensive and certain tsetse habitats are unsuitable for maintaining a small cattle population, (b) intense entomological monitoring using standard trapping devices. The level of confidence that there are no flies in the surveyed area will be determined by the number of traps deployed per km^2 and the time frame of the monitoring operation, (c) the release of large numbers of virgin gamma sterilised female tsetse flies. The introduction of large numbers of tsetse flies in the control or potentially cleared area, will bring the density of the fly population above the level of detection of the used trapping device. Assuming that one wild male fly can inseminate 2 to 6 sterile females successfully (Jordan, 1972; Van der Vloedt, personal communications), the technique in essence amplifies the wild male fly population. The same level of confidence that relic pockets of wild flies are absent will be obtained with far fewer traps and within a much shorter time frame as with option b. Aside from certain logistic advantages, the technique will permit tsetse control officers to respond quickly to exposed relic fly pockets and organise efficiently emergency mop-up operations. The feasibility of the sterile female release - recapture technique was demonstrated during a small field trial on the island of Unguja (Vreysen *et al.*, 1992). The test showed that (1) released sterile *G. austeni* female flies can be recaptured using standard trapping devices (sticky panels on Unguja), (2) sterile female flies were traced by the wild male flies with mating occurring from the second day after release, (3) sterile females survived very long in the field (host requirements is assumed to be low due to the absence of developing larvae resulting in the building-up of excess fat).

In their study on gamma sterilised *Glossina austeni* females, Vreysen & Van der Vloedt (1992) observed that a quantitative assessment of wild fly population densities using the release and recapture of sterile female flies is impossible. Due to their high multiple mating rate and the fact that members of the *morsitans* group do not display mating scars on the 6 th tergite, the method is limited to a straightforward exposure of a wild fly population. The situation with *G. tachinoides* is different. Being a member of the *palpalis* group, mating scars can easily reveal the occurrence of multiple matings. It makes it a potential candidate for further research to use the sterile female release technique for the measurement of the actual size of tsetse fly populations.

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Part 2 The effect of hybridisation of closely related *Glossina* species on the reproductive biology

Chapter 8

THE EFFECT OF INTERSUBSPECIFIC HYBRIDISATION AND GAMMA RADIATION ON THE REPRODUCTIVE BIOLOGY OF *Glossina palpalis palpalis* (Robineau-Desvoidy) AND *Glossina palpalis gambiensis* Vanderplank

Abstract

The closely related tsetse fly subspecies *Glossina palpalis palpalis* (Nigeria origin) and *Glossina palpalis gambiensis* (Burkina Faso origin) hybridise readily in the laboratory. Hybridised *G. p. palpalis* females produced less offspring than the parental intrasubspecific crosses. Adult emergence was below 70% with at least 78% being females. Most female hybrids were fertile whereas most of the male hybrids were sterile when backcrossed to the *G. p. palpalis* parental line. All F_1 males were capable of transferring a spermatophore but their mates rarely had sperm-impregnated spermathecae. Their testes rarely contained mature sperm; moreover, sperm, when present had low or no motility.

During laboratory cage tests with virgin females of both subspecies and either sexually mature male *G. p. palpalis* or *G. p. gambiensis*, there was no indication for selective mating. The same was true when gamma irradiated males (120 Gy treatment in air) were used. In the latter case complete sterility was induced causing embryonic arrest in all inseminated female mates. Consequently, in ratio tests with untreated virgin *G. p. palpalis* females, untreated *G. p. palpalis* males and an increasing number of irradiated *G. p. gambiensis* males, there was a gradual decrease in production of viable offspring.

The results of the present study are discussed with a view of using a combined hybridisation and induced sterility in distinct geographical zones where the two subspecies are present.

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Table 1. Fertility of intersubspecific crosses between *G.p.palpalis* and *G.p.gambiensis*

Female	Male	No. mature females	Female survival day 35 %	Female survival day 55 % [3]	Mating status MS +/SP + % [4]	Fecundity [5] day 35	Fecundity [5] day 55	Mean puparial weight ± SD (mg)	Emergence / Female %
Gpp	*Gpp*	190	96.6	89.3	100/ 98.8	0.093	0.086	33.2 ± 4.3	84.8/57.9
Gpg	*Gpg* (*)	200	91.0	84.3	no record	0.091	0.082	25.6 ± 4.1	90.7/49.8
Gpg [1]	*Gpp*	94	93.0	88.4	100/100	0.099	0.094	25.7 ± 3.8	94.8/56.7
Gpg [2]	*Gpp*	74	93.7	87.7	100/ 97.2	0.097	0.084	25.9 ± 3.7	95.7/53.9
Gpp	*Gpg* [1]	88	90.7	80.4	100/ 98.8	0.063	0.061	31.7 ± 5.6	69.1/78.9
Gpp	*Gpg* [2]	62	87.2	74.3	100/ 89.1	0.055	0.052	30.0 ± 4.4	46.2/92.8

1. *Gpg* from Bobo Dioulasso facility
2. *Gpg* from IEMVT(Burkina Faso origin)
* Data from CRTA, Bobo Dioulasso

3. Dynamic survival relative to mature female days
4. MS +: *Mating Scars present*
SP +: Spermathecae with motile sperm
5. No. pupae/mature female day

INTRODUCTION.

Hybridisation of closely related allopatric or sympatric tsetse fly species is a possible genetic mechanism for introducing sterility into a population for control. Most of the earlier work on hybridisation has been done on members of the *morsitans* group (Vanderplank, 1947; Curtis, 1972; Curtis *et al.*, 1980; Gooding, 1984; Gooding, 1987; Rawlings, 1985), but more recently the approach was extended to the *palpalis* group (Gouteux & Millet, 1984; Gooding, 1988). Two subspecies of the latter, *G. palpalis gambiensis (Gpg)* and *G. palpalis palpalis (Gpp)* are of particular interest in West Africa where they are the main vectors of trypanosomiasis.

This paper reports on the results of hybridisation experiments with *Gpp* and *Gpg* aimed at examining:

(1) the mating behaviour and fertilisation in intersubspecific crosses and in the backcross to the *Gpp* parental line,
(2) the level of induced sterility after intersubspecific mating with irradiated males (120 Gy treatment in air),
(3) the impact of irradiated *Gpg* males on the reproduction capacity of a *Gpp* population in laboratory ratio tests.

MATERIALS AND METHODS

Experimental flies

The flies used in this study were: *Gpp* originating from Nigeria and maintained on a membrane feeding system in the Entomology Unit, Seibersdorf laboratory since 1981 (Van der Vloedt *et al.*, 1987), and *Gpg* of Burkina Faso origin, received in 1988 as puparia from the rabbit-fed colony at IEMVT France as well as from the membrane-fed colony of the Bobo Dioulasso facility in Burkina Faso (Bauer *et al.*, 1984; Itard & Bauer, 1984). All experimental flies were kept under standard colony conditions at 24 ± 0.5 °C and 85 ± 5% RH and fed 6 times a week through a silicone rubber membrane on equal proportions of frozen and thawed porcine and bovine blood. *Gpg* flies from IEMVT were additionally fed on guinea pigs 2-3 times a week.

Table 2. Fertility of F1, F2 and F3 hybrids, when backcrossed to the *Gpp* parental line

Female	Male	No. mature females	Female survival day 35 % [1]	Mating status MS +/ SP+ % [2]	Mean puparial weight (mg) ± SD	Fecundity [3]	No. [4] aborted eggs & imm. larvae	Emergence / Female %
Gpg X Gpp	*Gpp*	61	90.7	98.4 / 91.9	25.1 ± 3.8	0.065	37	90.6 / 51.7
Gpp X Gpg	*Gpp*	86	97.9	98.8 / 98.8	27.4 ± 3.9	0.088	25	90.1 / 57.9
Gpp	*Gpg X Gpp*	49	98.3	98.1 / 7.8	29.7 ± 4.0	0.007	115	5/6 / 2/5
Gpp	*Gpp X Gpg*	35	96.3	100 / 0.0	-----	0.000	82	-----
(GpgXGpp)XGpp	*Gpp*	26	99.6	100 / 92.3	28.0 ± 3.2	0.069	8	93.1 / 59.3
(GppXGpg)XGpp	*Gpp*	101	97.7	98.1 / 93.5	27.2 ± 3.7	0.070	36	89.6 / 63.4
GppX(GpgXGpp)	*Gpp*	1	100	1/1 / 1/1	-----	0.111	0	2/2 / 1/2
Gpp	*(GpgXGpp)XGpp*	39	98.0	97.4 / 35.9	30.4 ± 4.1	0.035	62	83.3 / 70.0
Gpp	*(GppXGpg)XGpp*	91	98.7	98.9 / 44.0	30.7 ± 3.6	0.040	133	90.7 / 66.1
Gpp	*GppX(GpgXGpp)*	3	100	3/3 / 3/3	31.2 ± 2.9	0.093	0	4/5 / 1/4
[(GpgXGpp)XGpp]XGpp	*Gpp*	15	93.3	100 / 93.3	24.8 ± 5.1	0.071	8	82.3 / 42,8
[(GppXGpg)XGpp]XGpp	*Gpp*	59	94.4	100 / 98.3	28.4 ± 3.9	0.085	13	84.5 / 63.4
Gpp	*[(GpgXGpp)XGpp]XGpp*	11	100	100 / 72.7	32.4 ± 3.5	0.081	7	87.5 / 71.4
Gpp	*[(GppXGpg)XGpp]XGpp*	38	100	100 / 60.5	32.0 ± 3.4	0.053	47	80.5 / 55.2
GppX[(GpgXGpp)XGpp]	*Gpp*	12	100	100 /100	30.3 ± 2.8	0.106	0	90.9 / 55.0
GppX[(GppXGpg)XGpp]	*Gpp*	35	97.8	100 / 97.1	29.7 ± 4.5	0.083	15	88.2 / 41.2
Gpp	*GppX[(GpgXGpp)XGpp]*	7	100	100 / 57.1	31.8 ± 3.0	0.056	11	7/7 / 4/7
Gpp	*GppX[(GppXGpg)XGpp]*	19	96.2	100 / 68.4	31.7 ± 2.2	0.070	18	81.8 / 59.1

1. Dynamic survival relative to mature female days
2. MS +: Mating Scars present
   SP +: Spermathecae with motile sperm
3. No.pupae/mature female day
4. Observed

The crosses

In all crosses 2-3 day-old virgin female flies were mated with sexually mature males (at least 7-day old) at a 1:1 ratio. Flies of the intra and intersubspecific crosses were kept in standard colony cages and their performance monitored for 55 days. Backcrosses of F_1, F_2 and F_3 progeny with the *Gpp* parental line were kept individually in tubes (3.5 cm diameter, 6 cm high) and membrane-fed for an experimental period of 35 days. Daily checks were made for mortality, pupae production and occurrence of unsuccessful cycles. Female flies were dissected at the end of the experimental period and their insemination status and ovarian configuration determined. In all cross designations, maternal lines are given first and paternal second. In all experiments, fecundity is expressed as the number of puparia produced per mature female-day after taking day 18 following emergence as the first larviposition-day.

Sperm maturation and spermatophore formation

Respectively, 40 and 10 hybrid males resulting from crosses *(Gpg X Gpp)* and *(Gpp X Gpg)* were also individually mated with *Gpp* females to establish the mechanism of male hybrid sterility. The F_1 *(Gpp X Gpg)* males were given a second mating chance. Mating time was recorded and immediately after separation females were dissected and their uterus and spermathecae examined. The males were also dissected and squash preparations of the testes examined.

Mating preference and competitiveness tests

The potential mating preference of both males *Gpp* and *Gpg* was tested by exposing them to equal numbers of virgin females of both subspecies. Groups of 30 females were differently colour-marked and kept in plastic containers (20 cm diameter and 35 cm high) before 20 sexually mature males were introduced in lots of 10. The identity of the mating pairs was recorded; each experiment was replicated twice. The mating behaviour of irradiated males (120 Gy treatment in air) and untreated males of both subspecies was also assessed during competitiveness tests. Groups of 20 virgin *Gpp* females were exposed in the same container to equal numbers (2 x 30) of:

(1) untreated male *Gpp* and untreated male *Gpg*,
(2) untreated male *Gpp* and 120 Gy-treated male *Gpg* and
(3) 120-Gy treated male *Gpp* and untreated male *Gpg*.

Table 3. Spermatophore formation by F1 hybrid males and presence of sperm in testes

Female	Male	Average mating time min. ± SD	Females with spermatophore in uterus %	Females with mature sperm in spermatophore %	Females with mature sperm in spermathecae %	Males with mature sperm in testes %
Gpp	*Gpg X Gpp*	100.4 ± 25.8	100	7.5	5.0	23.0
Gpp	*Gpp X Gpg*	57.1 ± 29.3	100	0.0	0.0	33.3

Table 4. Mating preference of *Gpp* and *Gpg* males in presence of equal numbers of females of both subspecies [1]

Females	Males	Type of female observed *in copula*					
		Run I		Run II		Run III	
		Gpp	*Gpg*	*Gpp*	*Gpg*	*Gpp*	*Gpg*
Gpp *Gpg*	*Gpp*	10	10	10	10	9	11
Gpp *Gpg*	*Gpg*	10	10	10	10	8	12

1. 2 X 30 females in container, with 20 males being added.

Table 5. Receptivity of virgin *Gpp* females during competitiveness tests with equal numbers of *Gpp* and *Gpg* males on two consecutive days [1]

Type of male in test-container	No.	Day 1 Mating pairs and type of male *in copula*		Day 2 Mating pairs and type of male *in copula*	
		Gpp	*Gpg*	*Gpp*	*Gpg*
Gpp UT	26	13 UT	7 UT	5 UT	7 UT
Gpg UT	26				
Gpp UT	28	12 UT	8 T	9 UT	8 T
Gpg T	28				
Gpp T	27	8 T	12 UT	9 T	8 UT
Gpg UT	27				

1 . 20 females per test series

UT: untreated

T : treated (120 Gy in air)

The females were introduced in lots of 5; mating pairs were removed and the following day all females, after uncoupling, were re-exposed to the same males.

Intersubspecific crosses with gamma irradiated males

In order to determine the level of induced sterility as compared to the sterility obtained for an intrasubspecific control group, virgin females of one subspecies were mated with 120 Gy irradiated males of the other subspecies.

Ratio tests

For ratio tests, 200 virgin female *Gpp* were placed in each of eight 10 l containers (23). *Gpg* males were treated with 120 Gy in air when 4 - 5 days old. A total of 80 sexually mature untreated (UT) *Gpp* males and treated (T) *Gpg* males of the same age (8 - 9 days) were introduced into a container at the following female (UT): male (T) : male (UT) ratios: 200:0:80, 200:10:70, 200:20:60, 200:30:50, 200:40:40, 200:50:30, 200:70:10, 200:80:0. These flies were left together in the container for 6 days and fed daily on guinea pigs. After separation, females were transferred into normal holding cages in lots of 25 and fed *in vitro* stock colony diet. Female survival and pupae production was monitored for 45 days and the reproductive status of the females subsequently assessed by dissection.

RESULTS

Fecundity of the crosses.

Table 1 summarises the results of the hybridisation experiments. Striking are the high mating response, the resulting high mating scar rate (100 %) on the females' abdomen and the high insemination rate (at least 89 % in all groups). The fecundity in cross *Gpg X Gpp* was the only one that compared favourably with the results of cross *Gpp X Gpp* (0.094 and 0.084 versus 0.086 by day 55). Fecundity in replicates of the reciprocal cross *Gpp X Gpg* was reduced by 30% and 40% (0.061 and 0.052) by day 55. Moreover, the emergence rate of puparia from these crosses was only 69% and 46% as compared to 95% for puparia from cross *Gpg X Gpp*. Sex distortion in favour of the females (79% and 93%) was only found when *Gpp* was the mother.

Table 6. Fecundity and reproductive status of *Gpp* and *Gpg* females in intersubspecific matings with irradiated males (120 Gy in air)

Female	Male	No. initial females	Female survivors day 30 %	Mating status MS +/SP + % [1]	No. puparia produced	No. of aborted eggs [2]	No. of aborted imm. larvae	Uterus content(%) Recently ovulated egg	Degenerating egg	Immature larva L1	Immature larva L2	Immature larva L3	Uterus empty
Gpp	*Gpp*	50	96.0	100 /100	1	89	1	14.5	58.3	0.0	0.0	4.1	22.9
Gpg	*Gpp*	56	91.1	100 / 96.4	2	101	2	6.0	68.0	0.0	0.0	0.0	26.0
Gpp	*Gpg*	55	80.0	100 / 97.8	0	88	2	11.6	72.1	0.0	0.0	0.0	16.3

1. MS +: Mating Scars present
   SP +: Spermathecae with motile sperm
2. Observed

Data on backcrosses of the hybrids with the *Gpp* parental line are given in Table 2. As indicated by the insemination rate (at least 91%) F_1 female hybrids were equally receptive when backcrossed to *Gpp* males. Normal values were also obtained for emergence rate and sex ratio. Fecundity of the *(Gpp X Gpg)* females was comparable (0.088 by day 35) with that of the intraspecific *Gpp* control (0.093 by day 35) but a significant 30% reduction was noted for the *(Gpg X Gpp)* females. Females in the second and third backcross had a fecundity always exceeding 70% of the control with no obvious changes within successive generations.

The male progeny from cross *(Gpp X Gpg)* completely failed to inseminate *Gpp* parental females. Out of 53 hybrid males from cross *(Gpg X Gpp)* only 4 were able to successfully fertilise *Gpp* females; of the 6 pupae produced during the 35 day test period 5 were viable and produced fully fertile adult flies. F_2 males from cross *((GpgXGpp)XGpp)* and cross *((GppXGpg)XGpp)* were backcrossed with *Gpp* females and inseminated 36 and 44% of the females, respectively. The corresponding fecundity values were 0.035 and 0.040. A further increase in fertility was found with F_3 males. In all four backcross lines, female fecundity was at least 55% of the control.

Sperm maturation and spermatophore transfer by hybrid males

Table 3 contains dissection data for *Gpp* females mated with F1 hybrid males. In all cases, immediately after mating a spermathophore was found in the female uterus, but males from cross *(Gpg X Gpp)* on average needed twice as long to successfully complete the mating as males from the reciprocal cross. Examination of testes of*(Gpg X Gpp)* males and spermathophore content in mated females, revealed mature sperm in 23% of the males, whereas only 3 of 40 individually isolated spermatophores contained detectable amounts of mature sperm. In the *(Gpp X Gpg)* males and their female mates, the picture was even more aberrant with 33 % of the males having sperm in the testes, but with no sperm recovered from the spermatophores. In all the above cases, sperm when present, had little or no motility. Almost all the testes examined were saturated with fast turning cells lacking the characteristic flagellum. Moreover, testes of hybrid males were always markedly smaller than normal.

Table 7. Ratio tests with virgin *Gpp* females and treated (T) *Gpg* and untreated (UT) *Gpp* males

F : MT : MUT	No. mature females	Female survival day 45 %[1]	Mating status MS +/SP + % [2]	No. pupae produced	Fecundity [3]	Reduction in fecundity %
200: 0:80	192	84.6	98.1 / 96.3	452	0.096	0.0
200:10:70	195	85.0	98.0 / 98.4	388	0.082	14.7
200:20:60	194	87.3	99.0 / 94.8	277	0.057	40.7
200:30:50	187	82.3	95.4 / 93.1	248	0.054	43.7
200:40:40	186	86.1	99.5 / 96.2	178	0.037	61.4
200:50:30	182	80.0	95.5 / 94.9	135	0.030	68.5
200:70:10	192	85.5	100 / 95.3	88	0.018	80.8
200:80: 0	179	85.3	99.5 / 98.4	11	0.002	97.6

1. Dynamic survival relative to mature female days
2. MS +: Mating Scars present
   SP +: Spermathecae with motile sperm
3. No. pupae/mature female day

Mating preference and competitiveness

The results given in Table 4 indicate that *Gpp* and *Gpg* males show no mating preference for their own or related subspecies females in a mixed female population. Additionally, *Gpp* and *Gpg* females appeared equally receptive to both types of males. Table 5 also indicates that untreated males and sexually sterilised males of both subspecies were equally competitive for *Gpp* virgin females. The receptivity of these females was reduced at a second mating with no detectable preference to remate with either type of male.

Intersubspecific crosses with irradiated males

Table 6 indicates that mating occurred readily (i.e. all females had mating scars), with 96% *Gpg* females and 97% *Gpp* females being inseminated. Complete sterility was obtained in females of both subspecies. All *Gpg* females inseminated by irradiated *Gpp* males and all *Gpp* females inseminated by irradiated *Gpg* males expelled eggs with embryonic arrest. Dissection of the females on day 30 post - emergence, revealed an uterine content picture comparable with the one found in *Gpp* females mated with treated *Gpp* males (Van der Vloedt *et al.*, 1978). The majority of the females had a degenerating egg *in utero* or an empty uterus due to extrusion of the dead embryo. Almost no females were found in an advanced pregnancy stage.

Ratio tests

Results of cage tests with a *Gpp* population subjected to an increasing ratio of gamma treated *Gpg* males are given in Table 7. In all groups, female survival was high and there were no differences in insemination rates. A gradual increase in the number of irradiated *Gpg* males versus untreated *Gpp* males and females resulted in a gradual decrease in female fecundity. In the absence of treated *Gpg* males, the fecundity of the *Gpp* females after 45 days was 0.096. A male population consisting of 50% untreated *Gpp* and 50% treated *Gpg* males, reduced the fecundity of the *Gpp* females to 0.037 which represents a reduction of 61%. The presence of 100% treated *Gpg* and a 1:2.5 male/female ratio induced 97.6% sterility in the *Gpp* females.

The drastic changes in fecundity observed during ratio tests are also seen in the pregnancy dynamics of the females that were dissected on day 45 (Table 8). The uterus in control females (200:0:80) contained predominantly a healthy looking immature larva (71%) or an egg

Table 8. Reproductive status of *Gpp* females at termination of ratio tests

F : MT : MUT	200:0:80	200:10:70	200:20:60	200:30:50	200:40:40	200:50:30	200:70:10	200:80:0
No. inseminated females (LP age) [1]	183	184	184	176	179	168	182	165
No. inseminated females (day 45)	154	159	163	157	163	144	161	152
Fecundity [2]	0.096	0.082	0.057	0.054	0.037	0.030	0.018	0.002
Ovarian Configuration (%) [3]								
A cycle	84.2	78.1	64.3	60.0	59.1	62.8	33.8	36.2
C cycle	6.7	10.3	12.7	27.7	29.9	16.8	50.6	58.7
B cycle	6.7	4.1	0.6	3.1	0.6	4.4	3.2	0.7
D cycle	0.6	6.2	21.7	9.2	7.8	13.9	5.8	2.9
Determination not possible	1.8	1.3	0.6	0.0	2.6	2.2	6.5	1.4
Uterus content (%)								
Recently ovulated egg	21.4	29.1	30.2	28.9	21.1	19.4	6.9	9.2
First instar larva	17.9	16.5	9.9	6.3	7.5	4.9	1.9	0.7
Second instar larva	40.5	27.8	11.7	18.3	14.3	7.6	3.8	0.0
Third instar larva	12.7	10.7	19.8	9.1	6.2	13.2	2.5	0.7
Degenerating egg	0.0	6.3	9.9	19.7	29.2	16.7	44.4	51.6
Empty	7.5	9.5	18.5	17.6	21.7	38.2	40.6	37.9
Empty uterus due to (%)								
Abortion + Ovarian blockage	4.6	8.2	9.9	12.7	15.5	29.2	31.3	34.6

1. LP age = day 18 following emergence
2. No. pupae/ mature female day
3. Most advanced egg follicle in ovulation sequence A.C.B.D.

undergoing normal embryogenesis (21%). Evidence for an unsuccessful cycle was found in only 4.5% of the females. The results in the test series (200:40:40) and the 100% treatment group (200:80:0) are very indicative for the level of induced sterility and the impact on the reproductive status of the females with namely 28% and 1.4% immature larval stages, 14% and 33% abortion rates and 44.7% and 86.2% aberrations between ovarian development and uterine content, respectively. Taking the ovarian configuration system (Saunders, 1960; Challier, 1965) as a further basis for assessing the impact of various male ratios, it is also striking that the number of females undergoing acceleration of their ovarian cycle increased gradually as treated males increasingly outnumbered the fertile ones. This phenomenon is known and is indicative for recurrent unsuccessful or recurrent incomplete pregnancy cycles terminated during early embryogenesis (Curtis, 1968; Matolin & Van der Vloedt, 1982). The data in Table 8 show that on day 45, 84% of all control females (in series 200:0:80) had their most advanced egg follicle, as expected, in position A (second time in internal ovariole of right ovary) and 6.7% of the females in position C (second time in internal ovariole of left ovary), whereas in 36 % of the females in test group 200:80:0 follicle A was the next in ovulation sequence, with 59% having follicle C as being most developed. These findings substantiate the acceleration effect.

DISCUSSION

Machado's monograph (1954) on the systematics and geographical distribution of the *palpalis* species has encouraged a number of tsetse field workers (Challier *et al.*, 1983; Garms *et al.*, 1987; Gouteux & Millet, 1984; Nekpeni *et al.*, 1989) to make observations on the postulated occurrence of intermediate forms of the subspecies *G. p. palpalis* and *G. p. gambiensis* in West Africa. Available information on the distribution of the subspecies in Ivory Coast and Liberia, indicate that they have markedly different ecological requirements. *G. p. gambiensis* inhabits the river systems of the dry and humid savannah in the western part of West Africa whereas *G. p. palpalis* is confined to the forest galleries of the humid savannah and forest zones in the eastern part of West Africa (Challier *et al.*, 1983). They most likely have different host preferences and could play different roles in the transmission of human and animal trypanosomiasis.

Whereas Garms *et al.* (1987) did not find any indication for existence of intermediate forms in Liberia, Challier *et al.* (1983), Gouteux &

Millet (1984) and Nekpeni *et al.* (1989) reported intergradation based on morphometrical data from male specimen collected in a defined contact zone of the two subspecies in Ivory Coast. The authors refer to a very narrow hybridisation zone were hybrid sterility might be responsible for low population densities. The potential of hybridisation under field conditions is certainly substantiated by results of laboratory hybridisation experiments (Southern, 1980; Gouteux & Millet, 1984; Gooding, 1988), including our own data.

In discussing the practical relevance of their observations on the contact between the two subspecies and results of their hybridisation tests, Gouteux & Millet (1984) and Nekpeni *et al.* (1989) raised an interesting point by asking: "Que donnerait des lâchers de mâles *G. p. gambiensis* dans une zone à *G. p. palpalis* ? N'y aurait-il pas là également un moyen de lutte génétique aussi éfficace que la lutte par lâcher de mâles stériles?" These questions have incited us: (i) to re-examine mating behaviour and fertility in crosses between these two economically important subspecies and of their hybrids when backcrossed to the parental line and, (ii) to monitor under laboratory conditions the impact of sexually sterilised males on both separate and mixed female populations of the two subspecies.

Our data confirm that *G. p. palpalis* and *G. p. gambiensis* hybridise readily in laboratory cages. It is noteworthy that our observations indicate that disruption of the process of spermatogenesis, as referred to by Southern (1980), seems only partially responsible for the mechanism of hybrid sterility. Nevertheless, lack of sufficient amounts of sperm in most hybrid males from cross *(Gpg X Gpp)* (77%) as well as from cross *(Gpp X Gpg)* (67%) together with the observed subnormal sperm motility, drastically reduce their inseminating potential when backcrossed. This could be explored for control purpose. However, it should also be pointed out that in the relative rare cases of successful transfer of sperm by hybrid males, *in utero* development of larvae proceeds normally and results in fully fertile offspring.

From a practical viewpoint, our data on the apparent absence of a mating barrier in the two subspecies together with the lack of evidence of assortative mating give extra incentive for potential combined use of hybrid sterility and induced sterility. During pilot tsetse control campaigns in Burkina Faso (Politzar & Cuisance, 1984) and in Nigeria (Takken *et al.*, 1986) the SIT was successfully used for autocidal eradication of the subspecies referred to in the present study. Our data on random mating, full competitiveness of both radiation treated and untreated *Gpp* and *Gpg* males and their ability to inseminate both types of females indicate that the SIT for the subspecies under consideration

could be implemented in a dual-purpose manner. In other words, a target population of the two subspecies in a defined area could be eradicated by releasing sterile males of its own or closely related subspecies. The major advantage of such an approach would be that only one of the two subspecies need to be mass reared. From economic and logistic points of view, such an approach would endorse the validity of area-wide control (e.g. in the Black Volta basin) based on conventional suppression techniques and the release of sterile flies.

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Chapter 9

MORPHOLOGICAL CHARACTERISATION OF THE GENITAL ARMATURE OF MALE AND FEMALE HYBRIDS FROM CROSSES BETWEEN *Glossina palpalis palpalis* (Robineau-Desvoidy) AND *Glossina palpalis gambiensis* Vanderplank

Abstract

The two subspecies *Glossina palpalis palpalis* (*Gpp*)(Robineau-Desvoidy) originating from Nigeria and *Glossina palpalis gambiensis* (*Gpg*) Vanderplank originating from Burkina Faso, could be completely separated morphometrically based upon the width of the terminal dilatations of the male inferior claspers. Intermediate values were obtained for the male hybrids, but the average size of the head of the parameres was significantly determined by maternal descendance i.e. the average width of the head of the inferior claspers of male hybrids from cross (*Gpg* x *Gpp*) was significantly larger than the one of hybrids from the reciprocal cross. In addition, distinct morphological characters of the inferior claspers are described for each of the male hybrids.

The dorsal plates of the genital armature of female *Gpg* were significantly longer but significantly less wide as compared to the dorsal plates of female *Gpp*. Morphometrical analysis based upon the length and width of the dorsal plates of the two species revealed only a minimal overlap (7%). Intermediate values were obtained for the length of dorsal and anal plates of female hybrids, but not for the width of the dorsal plates. The most optimal separation of female hybrids was obtained by plotting the length of the dorsal plates against the length of the anal plates (18% overlap).

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Table 1. Number of macrotrichae and widths of the terminal dilatations of the inferior claspers of male *G.p.palpalis*, *G.p.gambiensis* and their male hybrids

Female	Male	Males	Width (µm) of terminal dilatations (± SD)		No. macrotrichae on terminal dilatations (%)						Macrotrichae on body of inf.clasp.
		no.	Average (*)	Range	1	2	3	4	5	6	no. (*)
Gpp	*Gpp*	82	98.5 ± 8 a	78.8 - 116.1	4.2	40.3	48.4	10.5	0.0	0.0	56.5 ± 7.3 a
Gpg	*Gpg*	64	171.0 ± 10 b	138.8 - 190.1	0.0	8.8	39.5	46.5	4.4	0.9	56.9 ± 6.2 a
Gpp	*Gpg*	33	128.5 ± 10 c	101.0 - 147.1	0.0	14.5	51.6	32.3	1.6	0.0	56.4 ± 4.1 a
Gpg	*Gpp*	77	144.7 ± 10 d	117.8 - 165.2	0.0	8.6	59.7	28.1	3.6	0.0	55.9 ± 5.5 a

(*) Data in same column followed by a common letter are not significantly different ($p > 0.05$, Student's *t* test)

INTRODUCTION

The two closely related subspecies *Glossina palpalis palpalis (Gpp)* and *Glossina palpalis gambiensis (Gpg)* are the predominant vectors of animal and human trypanosomiasis in West - Africa (Buxton, 1955). The two subspecies occur in separate geographic regions of West - Africa, except for a small area in the Ivory Coast, where they occur sympatrically (Challier *et al.*, 1983). They were described and classified by Vanderplank (1949) as two distinct subspecies based upon results of cross-breeding experiments and morphological analysis of the parameres of the male flies. The taxonomic status of the two subspecies was later confirmed by Machado (1954). The high hybridisation capacity of the two subspecies under laboratory conditions and the resulting male hybrid sterility has been well studied (Southern, 1980, Gooding, 1988, Vreysen & Van der Vloedt, 1990). Indirect evidence for inter-subspecific matings has been found in the Ivory Coast based upon the intermediate values for the width of the terminal dilatations of the inferior claspers (Challier *et al.*, 1983: Gouteux & Millet, 1984; Nekpeni *et al.*, 1989). No detailed morphological description of the external genitals of both female and male hybrids is however available. The results of a morphometrical study of the inferior claspers and the genital plates of male and female hybrids resulting from laboratory crosses of *Gpp* and *Gpg* are presented in this paper.

MATERIAL AND METHODS

The *Gpp* and *Gpg* parental lines originated from Kaduna (Nigeria) and Bobo Dioulasso (Burkina Faso) respectively. The hybrid flies were derived from cross breeding experiments as described in chapter 8. In all cross designations, maternal lines are given first, paternal second. Permanent preparations were made of the inferior claspers of the males of the parental lines and of the resulting hybrids. The complete hypopygium was removed from the male flies' abdomen and cleared overnight in warm 10% potassium hydroxide and then transferred for 5 - 10 minutes to glacial 100% acetic acid. The inferior claspers were removed from the hypopygium under binocular and mounted in Euparal. Measurements of the terminal dilatations of the head of the inferior claspers were made under a Leitz compound microscope at x 64 magnification. For each male, the average value of the two inferior

claspers was used for the analysis. The number of macrotrichae or bristles on the body of the inferior claspers was counted on photographs taken with the Leitz compound microscope. The same technique was used to make permanent preparations of the external genitals i.e. the dorsal and anal plates of female *Gpg* and *Gpp* and female hybrids resulting from the crosses. Maceration in warm 10% potassium hydroxide was limited to 15 - 30 minutes to prevent the dorsal and anal plates becoming completely translucent. Measurements were made of the length and the width of the dorsal plates and the length of the anal plates. Discriminant analysis (Statgraphics 4.0) was used to discriminate between the two subspecies and their hybrids using the variables length and width of the dorsal and anal plates. The average value of the left and right dorsal and anal plates of each female was used in the analysis.

RESULTS

A. Male genital armature

1. Biometrical analysis

The inferior claspers of 82 male *Gpp*, 64 male *Gpg*, 33 male hybrids resulting from cross (*Gpp* x *Gpg*) and 77 male hybrids resulting from cross (*Gpg* x *Gpp*) were examined (Table 1). The mean width of the terminal dilatations of the male inferior claspers was 98.5 ± 8.1 µm (range 78.8 - 116.1 µm) and 171.0 ± 9.5 µm (range 138.8 - 190.1 µm) for *Gpp* and *Gpg* respectively ($p < 0.001$). Measurements of the heads of the inferior claspers of male hybrids revealed intermediate values with averages of 128.5 ± 9.9 µm (range 101.0 - 147.1 µm) and 144.7 ± 10.3 µm (range 117.8 - 165.2 µm) for the hybrids from cross (*Gpp* x *Gpg*) and cross (*Gpg* x *Gpp*) respectively ($p < 0.001$). The frequency distribution of the different size classes of the terminal dilatations of the inferior claspers is presented in Fig. 1. This permitted a complete separation between the two subspecies, whereas only a minimal overlap was observed between the various size classes of male *Gpp* and the hybrids from cross (*Gpg* x *Gpp*) (n = 2, 1.2%) and male *Gpg* and the hybrids from cross (*Gpp* x *Gpg*) (n = 1, 1.0%).

The number of macrotrichae or apical bristles on the terminal dilatations of the inferior claspers was limited to 2 (40.3%) or 3 (48.4%) in 88.7% of the examined *Gpp* males. The majority of *Gpg* males however, were found with 3 (39.5%) or 4 (46.5%) apical bristles. 4.2% of

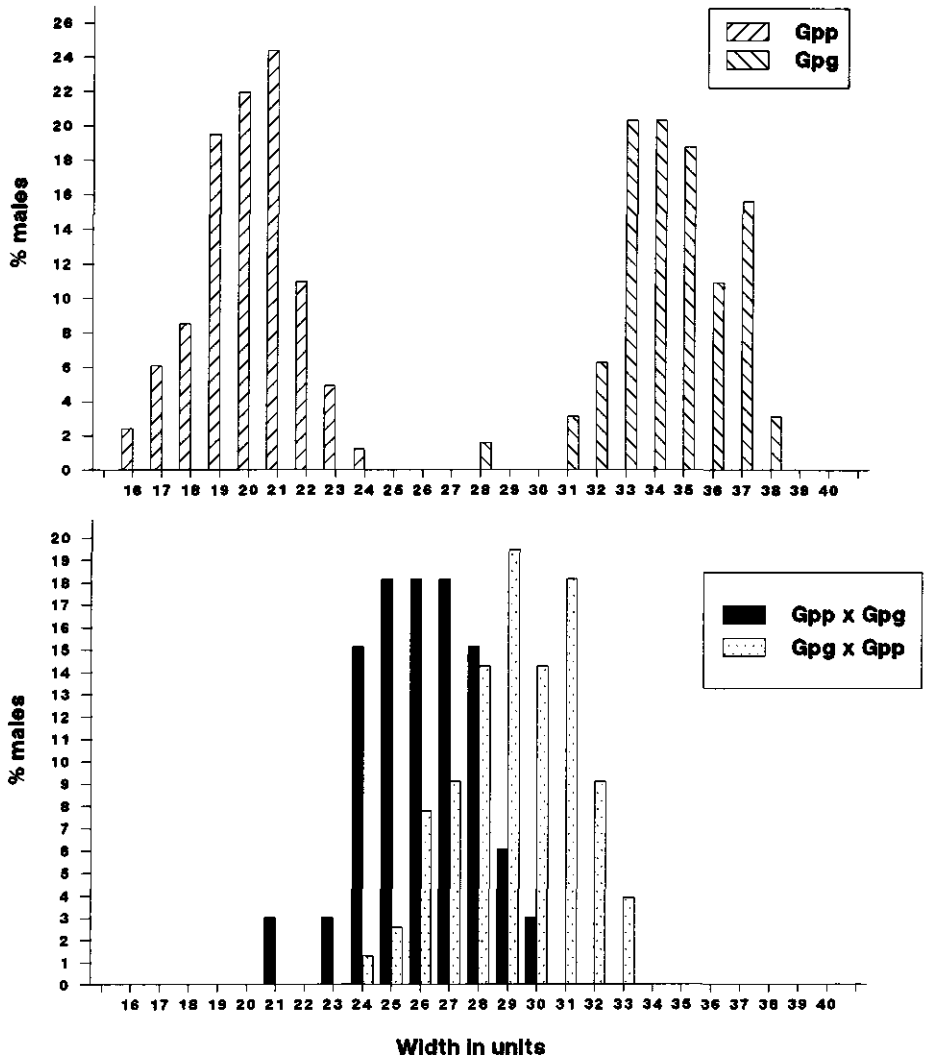

Fig. 1 Frequency distribution of different size classes of the width of the terminal dilatation of the interior claspers of male *G. p. palpalis* , *G. p. gambiensis* (top) and their hybrids (bottom) (1 unit = 5 µm).

the *Gpp* males had only 1 bristle whereas 5.3% of the *Gpg* males revealed 5 or 6 macrotrichae. No differences were observed for either hybrids with 83.9% and 87.8% of the males of cross (*Gpp* x *Gpg*) and cross (*Gpg* x *Gpp*) respectively with 3 or 4 macrotrichae (Table 1). In addition, no differences could be detected with respect to the number of macrotrichae on the body of the inferior claspers with an average number of 56.5 ± 7.3, 56.9 ± 6.2, 55.9 ± 5.5 and 56.4 ± 4.1 for male *Gpp*, *Gpg*, males from cross (*Gpg* x *Gpp*) and from cross (*Gpp* x *Gpg*) respectively.

2. Morphological aspects

The general morphology of the inferior claspers of both subspecies has been described in detail by Machado (1954). Plate 1 shows the general morphological features of the inferior claspers of the *Gpp* and *Gpg* parental lines used in the laboratory crosses. The body of the inferior claspers of *Gpp* of Nigerian origin, was characterised by (1) parallel internal and external borderlines, (2) a neck who has the same width over its entire length, (3) an internal border of the body making a straight line, (4) absence of an internal hunk but a pronounced external hunk with tangents making an angle of approximately 90°, (5) narrow terminal dilatations with the internal lobe very little pronounced and the external lobe varying from a round structure to a larger "hammer" like character. The body of the inferior claspers of *Gpg* of Burkina Faso origin was characterised by (1) the inner side of the body being bent externally, resulting in non-parallel internal and external borderlines, (2) the neck of the clasper narrows gradually towards the terminal dilatation making the external hunk less prominent as in *Gpp*, (3) a small internal hunk apparent at the base of the body and (4) the large terminal dilatations have a very pronounced external lobe.

Marked differences were observed in the general morphological appearance of the inferior claspers of the hybrids depending on the cross:

A. HYBRIDS FROM CROSS GPP X GPG

Inferior claspers of male hybrids resulting from crosses of (*Gpp* x *Gpg*) were characterised by (1) the internal and external lobes of the terminal dilatations equal in size, (2) an internal border which is only slightly bent, (3) a very prominent external hunk, with at the base a depression or internal indentation, (4) indentation of the terminal

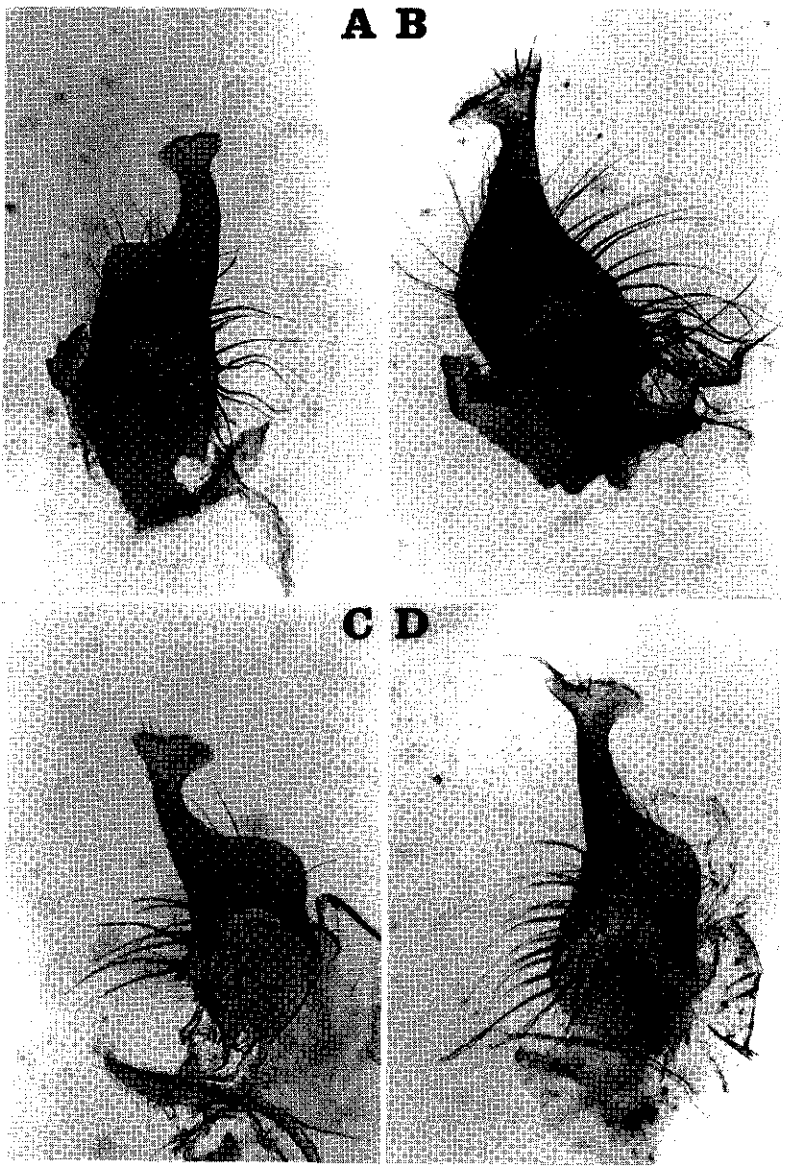

Plate 1. Diagrams of male inferior claspers. A: *Glossina palpalis palpalis*, B: *Glossina palpalis gambiensis*, C: *Gpp* x *Gpg*, D: *Gpg* x *Gpp*.

dilatation present but less prominent as in the hybrids from the cross *Gpg* x *Gpp*. (Plate 1 C)

B. HYBRIDS FROM CROSS GPG X GP P

Hybrid males from the reciprocal cross had inferior claspers with (1) a "hammer" like terminal dilatation with the external lobe being larger than the internal lobe, (2) a bent internal borderline (with less inclination as in *Gpp*) resulting in an internal hunk, (3) a prominent external hunk (more prominent as in *Gpg*) but with considerable variation in size, (4) the presence of an indentation at the distal end of the terminal dilatation (not present in *Gpg*). (Plate 1 D)

B. Female genital armature

Measurements of the left and right dorsal plates of female *Gpp* revealed an average length of 362.8 ± 22.4 µm and an average width of 340.3 ± 20.1 µm (n = 74) (Table 2). Female *Gpg* (n = 69) displayed dorsal plates which were on average significantly longer (427.2 ± 24.4 µm, $p < 0.001$) and less wide (321.2 ± 19.7 µm, $p < 0.001$). The average length (420.9 ± 21.2 µm) and width (346.9 ± 22.5 µm) of the left and right dorsal plates of female hybrids resulting from cross (*Gpp* x *Gpg*) was not significantly different from the length of the dorsal plates of the female *Gpg* parental line and from the width of the dorsal plates of the female *Gpp* parental line. The average length of the dorsal plates of the reciprocal cross (*Gpg* x *Gpp*) (374.6 ± 31.5 µm) was comparable to the length of those of female *Gpp*, whereas the average width (313.6 ± 26.5 µm) was not significantly different from the one observed for the dorsal plates of female *Gpg*.

Analysis of the length of the anal plates revealed significant differences between the two subspecies ($p < 0.001$, average length of 248.5 ± 14.4 µm and 280.4 ± 19.0 µm for *Gpp* and *Gpg* respectively), between *Gpp* and female hybrids from the two crosses (average length of 275.7 ± 21.9 µm and 258.3 ± 16.6 µm for female (*Gpp* x *Gpg*) and for female (*Gpg* x *Gpp*) respectively) and between female *Gpg* and females resulting from cross (*Gpg* x *Gpp*).

The length of the dorsal plates plotted against the width of the dorsal plates is presented in Fig. 2 (top). Discriminant analysis revealed a nearly complete separation of *Gpp* and *Gpg* females with only 11 specimens not identifiable as either *Gpp* or *Gpg* (7.7% overlap). Characters overlapped more for females resulting from the crosses (18.7%) (Fig. 2, bottom graph). A significant correlation was revealed

Table 2. Average dimensions of the dorsal and anal plates of female *G. p. palpalis, G. p. gambiensis* and their hybrids

Female	Male	Females no.	Average dimensions (± SD) dorsal plates (in µm) height (*)		Average dimensions (± SD) dorsal plates (in µm) width (*)		Average height (± SD) anal plates (in µm) (*)	
Gpp	*Gpp*	74	362.8 ± 22.4	a	340.3 ± 20.1	a	248.5 ± 14.4	a
Gpg	*Gpg*	69	427.2 ± 24.4	b	321.2 ± 19.7	b	280.4 ± 19.0	b
Gpp	*Gpg*	30	420.9 ± 21.2	b	346.5 ± 22.5	a	275.7 ± 21.9	b
Gpg	*Gpp*	32	374.6 ± 31.5	a	313.6 ± 26.5	b	258.3 ± 16.6	c

(*) Data in the same column followed by a common letter are not significantly different ($p > 0.05$, Student's t-test)

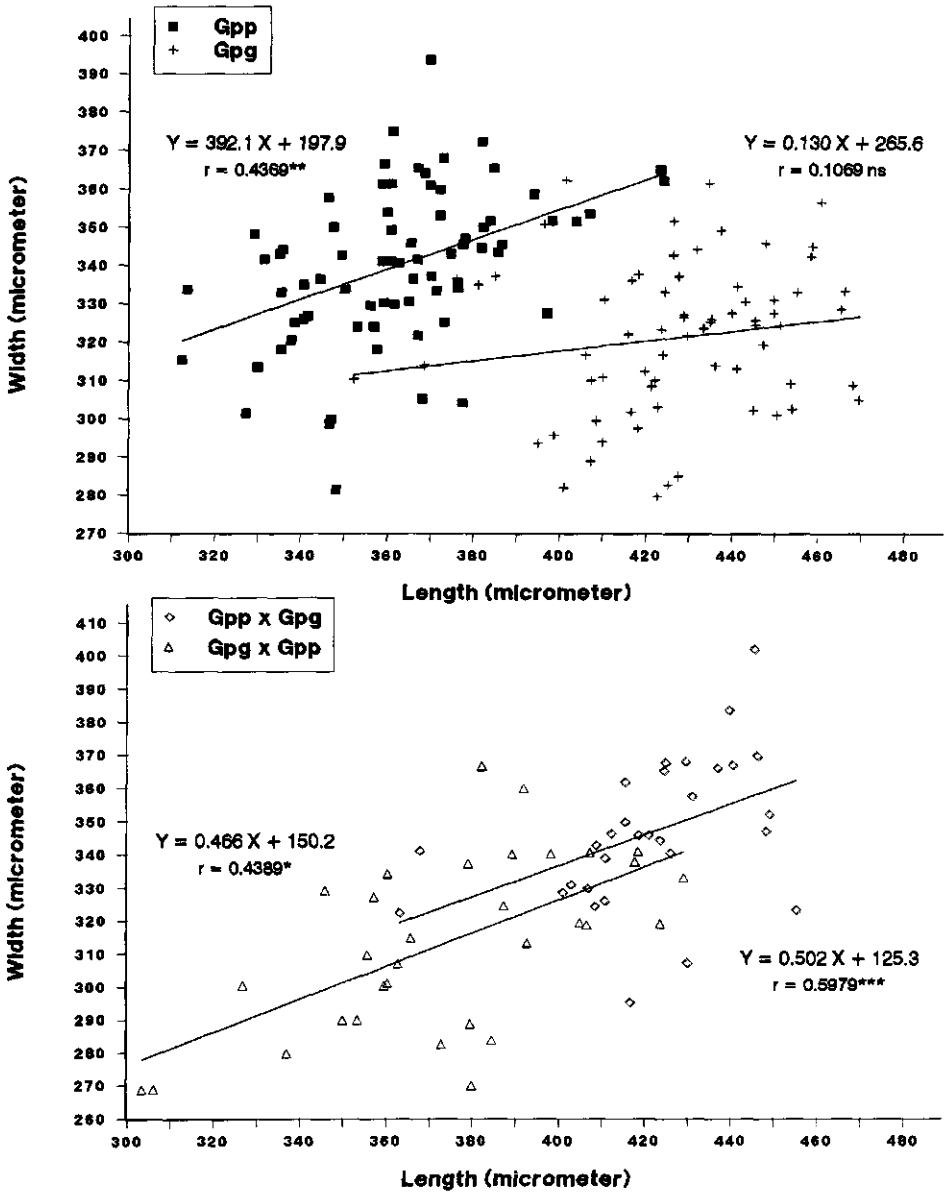

Fig. 2 The length of the genital dorsal plates plotted against the width of the dorsal plates of female *Gpp* and *Gpg* (top graph) and their female hybrids (bottom graph) (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns = not significant).

between the length and width of the dorsal plates of female *Gpp* ($r = 0.4369$, $p < 0.01$), female (*Gpp* x *Gpg*) ($r = 0.4389$, $p < 0.05$) and female (*Gpg* x *Gpp*) ($r = 0.5979$, $p < 0.001$) but not for female *Gpg* ($r = 0.1069$, $p > 0.05$). The correlation between the length of the dorsal plates and the length of the anal plates is shown in Fig. 3. The same overlap in characters was found for the two subspecies (7.2% or 10 specimen could not be identified) but more for the female hybrids (20%). The length of the dorsal plates was not significantly correlated with the length of the anal plates for female *Gpp* ($r = 0.2262$, $p > 0.05$), female *Gpg* ($r = 0.015$, $p > 0.05$) and for female (*Gpp* x *Gpg*) ($r = 0.1427$, $p > 0.05$). Females of the cross (*Gpg* x *Gpp*) revealed a positive correlation between the length of the dorsal and the anal plates ($r = 0.5543$, $p < 0.01$).

DISCUSSION

Males and females of the two closely related subspecies *Gpp* and *Gpg* mate readily under laboratory conditions. (Southern,1980; Gouteux & Millet,1984; Gooding, 1988; Vreysen & Van der Vloedt, 1990). The apparent absence of assortative mating was confirmed during laboratory cage tests with virgin females and sexually mature males of the two subspecies (chapter 8). The resulting hybrid male sterility has been attributed to an interruption of the spermatogenesis process (Southern, 1980) and to the low or complete absence of motility of sparsely produced mature hybrid sperm (chapter 8).

Several surveys have been carried out in West Africa to outline the geographical distribution of the two subspecies. All morphometrical characterisation of the sampled specimens was based upon the width of the terminal dilatation of the inferior claspers of the male flies i.e. the only character reliable for diagnostic purposes (Vanderplank, 1949). Our measurements of the parameres of male *Gpp* of Nigerian origin and *Gpg* of Burkina Faso origin, permitted a complete separation of the two subspecies and confirmed observations made by Challier *et al.* (1983), Gouteux & Millet (1985), Garms *et al.* (1987) and Nekpeni *et al.* (1989). The occurrence of hybrids in nature has remained more enigmatic due to the lack of a detailed description of hybrid morphology. Machado (1954) alludes to the existence of specimens with intermediate morphological characters in Liberia, Ghana and Ivory Coast and considered the entire south of the latter as a large zone of hybridisation. These findings were questioned by Gouteux & Dagnogo (1985) who point out the existence of

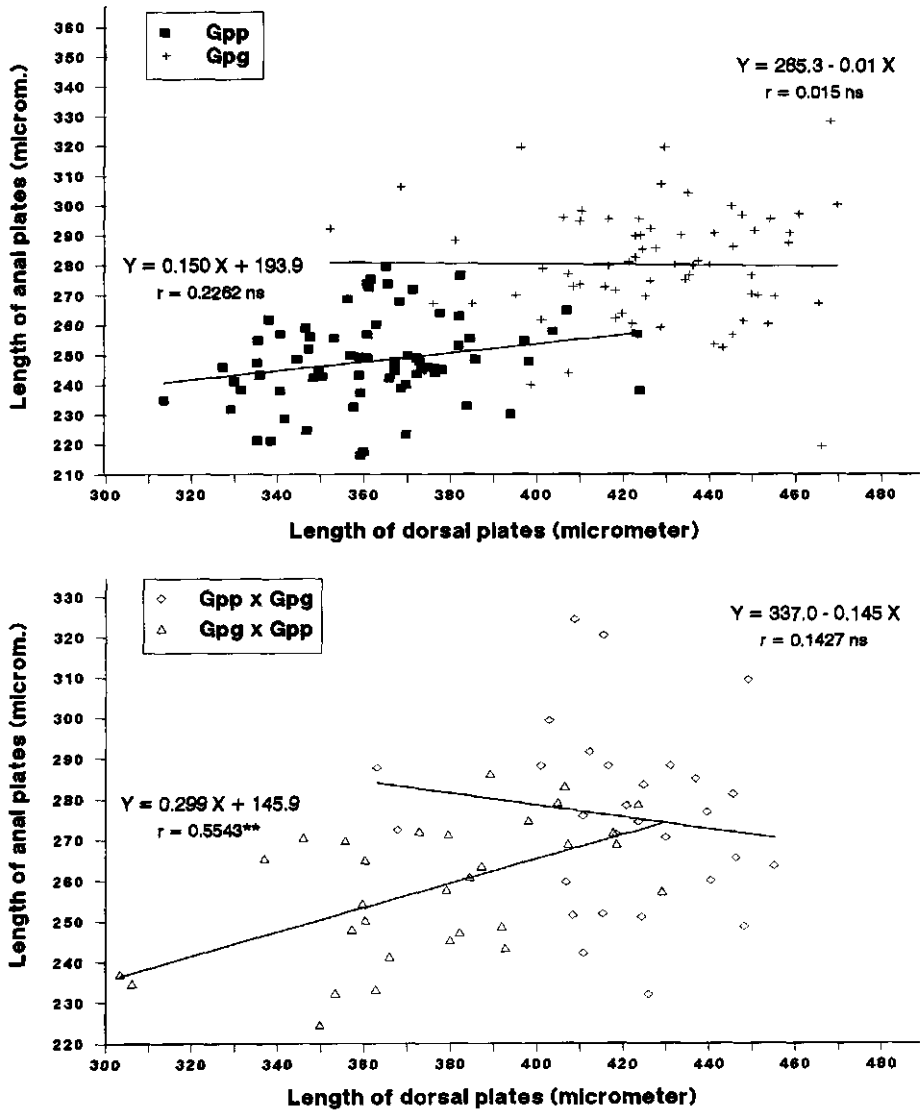

Fig. 3 The length of the genital dorsal plates plotted against the length of the genital anal plates of female *Gpp* and *Gpg* (top graph) and their female hybrids (bottom graph) (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns = not significant).

considerable morphological variation in the length of the head of the inferior claspers of male flies sampled in the southern part of Ivory Coast; statistical analysis revealed a homogeneous *Gpp* population. Likewise, no evidence of intergradation was found in Liberia (Garms *et al.* ,1987). However, a few specimens with intermediate values for the width of the terminal dilatations of the inferior claspers were sampled during surveys carried out in a narrow contact zone of the two subspecies in Ivory Coast (Challier *et al.,* 1983; Gouteux & Millet, 1984; Nekpeni *et al.*, 1989). These field data indicated (1) the apparent absence of ethological mating barriers between the two subspecies, (2) the occurrence of intersubspecific matings in nature resulting in (3) offspring with intermediate morphological characters in a very narrow hybridisation zone. These survey results were substantiated by a morphometrical analysis of the parameres of 13 hybrid males resulting from a laboratory cross of female *Gpg* and male *Gpp* (Gouteux & Millet, 1984). Our morphometrical data of male hybrids resulting from both crosses corroborate the data of Gouteux & Millet (1984) i.e. intermediate values for the length of the head of the parameres were revealed for male hybrids of both crosses but significant differences were obtained depending on the maternal descendance. Moreover, distinct morphological characters of the inferior claspers of male hybrids could be distinguished depending on the cross and have been described.

Furthermore, our data have indicated that also female flies of the two subspecies could be separated with a minimal overlap (7%) using morphometrical data of the dorsal plates of the genital armature. This is a better separation than the data of Garms *et al.* (1987) who states a 12% overlap of female characters (n = 105 females). Characters of the female hybrids overlapped more (between 18 - 20%).

The morphological characterisation of male and female hybrids resulting from crosses of *Gpp* and *Gpg* substantiate previous assumptions that male and female hybrids exhibit intermediate morphological characters and that cross mating does occur in nature. However, the hybrid morphology described in this paper is derived from hybrids descending from parental lines of two distinct geographical areas (Nigeria and Burkina Faso). It would be worthwhile to expand this study and analyse the reproductive parameters and characterise morphometrically the hybrids from sub-species originating from two neighbouring localities.

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Chapter 10

HYBRIDISATION OF THE TSETSE FLY SPECIES *Glossina palpalis palpalis* (Robineau-Desvoidy) AND *Glossina fuscipes fuscipes* Newstead

Abstract

Cross breeding experiments between the two species *Glossina palpalis palpalis (Gpp)* and *Glossina fuscipes fuscipes (Gff)* carried out in the laboratory revealed good mating response in the interspecific crosses (> 95% mating scars). Fertility in the cross (*Gpp* x *Gff*) was reduced with 50% as compared with fertility of the intra-specific *Gpp* cross. Emergence rate of pupae produced by this cross was reduced (60%) and all hybrid offspring were females. In the reciprocal cross (*Gff* x *Gpp*), the majority of the females (72%) were killed in the days following the mating due to piercing of the females' abdomen by the superior claspers of the *Gpp* males.

During experiments carried out in large laboratory cages (200 x 180 x 20 cm), no evidence of assortative mating was found between the two species.

The results of these experiments indicate the possibility of controlling *Gff* populations by the release of *Gpp* males, with the majority of *Gff* females killed as a result of the mating.

INTRODUCTION

The perception of introducing sterility in a tsetse fly population through hybridisation of closely related allopatric or sympatric subspecies was already conceived before the Sterile Insect Technique (SIT) concept was formulated (Knipling, 1963) or tested under field conditions (Jackson, 1945; Vanderplank, 1947). After the original work of Corson (1932) and Potts (1944) who obtained hybrids by crossing *G. morsitans* with *G. swynnertoni*, the mating competitiveness between the species was tested in a first field trial (Jackson, 1945). Extensive cross breeding experiments for various taxa of the *morsitans* and *palpalis* group were later carried out by Vanderplank (1948). Recently, more in-depth cross breeding studies with *G. morsitans morsitans*, *Glossina morsitans centralis* and *G. m. orientalis* confirmed the partial sterility between the crosses and the male hybrid sterility (Curtis, 1972; Curtis *et al.*, 1980; Gooding 1987; Rawlings, 1985). Incompatibility between the genes of gravid females and the hybrid larvae were considered to be the cause of the partial sterility (Curtis, 1972). A sperm defect preventing normal migration from spermatophore to spermathecae was attributed to cause the male hybrid sterility. Cross breeding experiments between two members of the *palpalis* group i.e. *Glossina palpalis palpalis* and *Glossina palpalis gambiensis* revealed (1) the high hybridisation capacity of the two subspecies, (2) the absence of any assortative mating, (3) almost complete sterility of the male hybrids (Gooding, 1988; Gouteux & Millet, 1984; Vreysen & Van der Vloedt, 1990).

Apart from the initial studies by Vanderplank (1948), no information is available about fertility of crosses between other members of the palpalis group (*G. p. palpalis*, *G. f. fuscipes*, *G. f. quanzensis* and *G. f. martinii)*. The present study intends to contribute to a wider understanding of the possibility of hybridisation of tsetse species for control purposes. The study presented here had two objectives: (1) to assess the fertility of the crosses between *G. palpalis palpalis* and *G. fuscipes fuscipes* and (2) to determine the extent of interspecific mating in large laboratory cages .

MATERIAL AND METHODS

The experimental flies were derived from following stock colonies of the Entomology Unit, Seibersdorf:

Table 1. Fertility of interspecific crosses between *Glossina palpalis palpalis* and *Glossina fuscipes fuscipes*

Female	Male	Initial females no.	Female survival		Mating status MS +/SP+ % [2]	Pupae produced no.	Mean puparial weight (mg) ± SD	Fecundity [3]	Emergence/ Females %
			LP age % [1]	day 55 %					
Gpp	*Gpp**	300	100	94.0	97.6 / 97.0	912	32.8 ± 3.9	3.04	95.6 / 52.3
Gff	*Gff**	95	94.7	75.8	96.8 / 93.6	219	31.1 ± 5.1	2.30	94.9 / 50.2
Gpp	*Gff*	100	94.0	83.0	98.8 / 94.0	147	28.8 ± 5.2	1.47	60.5 / 100
Gff	*Gpp*	90	27.7	17.7	95.9 / 76.1	10	34.0 ± 8.1	0.11	92.8 / 78.6

1. Larviposition day (ca. day 18)
2. MS +: Mating Scars present
   SP +: Spermathecae impregnated
3. No. pupae per initial female
* Data: Van der Vloedt (pers. comm.)

1. *Glossina palpalis palpalis*, originating from Kaduna, Nigeria and maintained since 1974 as an *in vivo* colony and since 1984 exclusively on an *in vitro* membrane feeding system (Wetzel & Luger, 1978)

2. *Glossina fuscipes fuscipes*, originated from the Central African Republic and introduced at the Seibersdorf Laboratory in 1986 from pupae supplied by I.L.R.A.D. (Nairobi).

All experimental flies were maintained in the insectaries together with the stock colonies under standard holding conditions (Van der Vloedt *et al.*, 1987). Flies were fed 6 times a week through a silicone membrane (Wetzel & Luger, 1978; Bauer *et al.*, 1984) on equal proportions of frozen and thawed porcine and bovine blood .

In all crosses, virgin females were mated on the second or third day after emergence with sexually mature males (> 6 days) in standard colony cages (diameter 10.5 cm, height 5 cm) at a 1:1 mating ratio. Reproductive parameters and survival were monitored for 55 days as described by Vreysen & Van der Vloedt (1990). In the cross designations, maternal lines are given first.

During a laboratory cage test, 100 virgin *Gff* females (3 days old) were placed in a large laboratory cage (dimensions 200 x 180 x 20 cm) after they were offered a blood meal. Equal numbers (n = 50) of sexually mature *Gpp* and *Gff* males of the same age (9 days) were differently colour marked on the thorax and after feeding, introduced in the large laboratory cage. Female flies were removed from the cage when all flies had died. Permanent preparations of the mating scars were made and measurements were carried out of the length, width and distance between the centres of the mating scars (chapter 11). Each individual female fly was classified as having mated with a *Gpp* or *Gff* male based upon the discriminant function derived from the mating scar analysis described in chapter 11 (Discriminant analysis, SAS for Windows). The test was replicated with 100 virgin *Gpp* females and equal numbers of sexually mature *Gpp* and *Gff* males.

RESULTS

The crosses

The results of the crosses are summarised in Table 1. Survival and fecundity of the *Gpp* intraspecific crosses was better as compared to the one of the *Gff* cross i.e. female survival of 94.0% versus 75.8% on

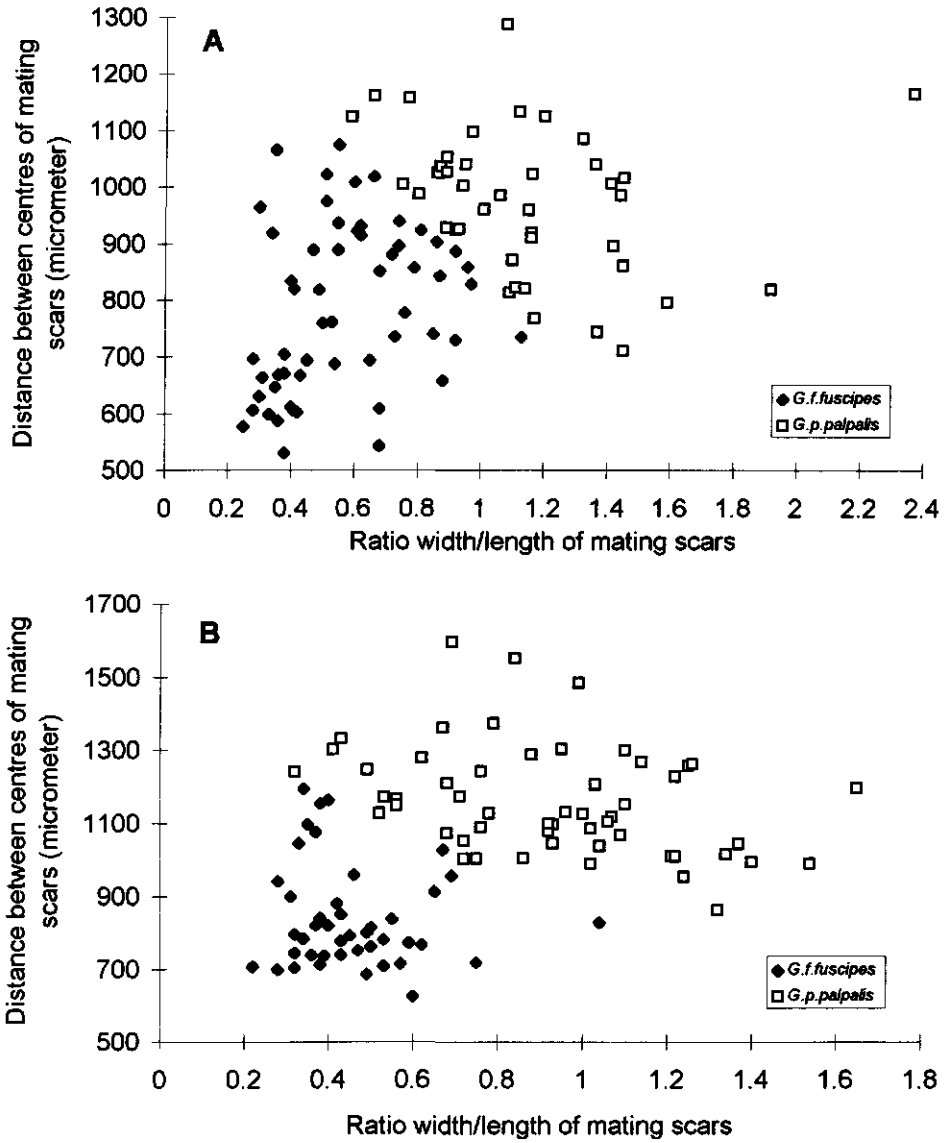

Fig. 1 Scatter diagram of the ratio width/length of the mating scars plotted against the distance between the centres of the mating scars of female *Gpp* (top) and female *Gff* (bottom) mated with male *Gpp* and *Gff* in large laboratory cages

day 55 and a fecundity of 3.04 versus 2.30 pupae per initial female for *Gpp* and *Gff* respectively. Emergence of the produced pupae was for both parental lines above 94% with 50 to 52% being females. Mating response in the interspecific cross *(Gpp x Gff)* was good (98.8% showing mating scars) with 94% of the *Gpp* females being inseminated.

Fecundity, expressed as the number of viable pupae per initial female was reduced with 52% as compared to the intraspecific crosses i.e. 1.47 versus 3.04 pupae per initial female. Average puparial weight (28.8 ± 5.2 mg) was significantly lower ($p < 0.001$) as compared to the weight of the pupae produced in the intraspecific cross (32.8 ± 3.9 mg). Only 60.5% of the produced pupae emerged and all offspring were females.

Mating response in the reciprocal cross (*Gff x Gpp*) was equally high (95.9% mating scars) but the *Gpp* males failed to inseminate 24% of the *Gff* females. Of the 90 *Gff* females mated on the second day of their adult life, only 27.7% and 17.7% were alive on day 18 and on day 55 respectively. Examination of the dead females revealed pierced abdomens and the high post-mating blood mortality was indicative for perforation of the mid gut. Only 10 pupae were produced by the surviving *Gff* females giving a fecundity of 0.11 pupae per initial female. Average puparial weight was significantly higher than the average weight of pupae produced by the reciprocal cross but comparable with the weight of pupae produced by the intraspecific controls. Emergence was normal (92.8%) and 78.6% of the offspring were females.

Large cage experiments

Fig. 1 and 2 present the data of the large cage experiment. Of the 100 female *Gff* and *Gpp* introduced in the large cage in the two experiments, 97 and 99 permanent preparations were made of their mating scars. Mating scars analysis (Chapter 11) revealed that 44.3% of the *Gff* females had mated with males of the same subspecies whereas 55.6% of the *Gff* females showed mating scars belonging to a mating with a *Gpp* male. Nine females showed double mating scars. In the second experiment, 58.6% of the *Gpp* females had mated with *Gff* males and 41.4% with *Gpp* males. Double mating scars were found in 7 females.

DISCUSSION

The hybridisation potential between the two closely related subspecies *Gpp* and *Gpg* , both belonging to the *palpalis* group, has been the subject

of various recent studies (Southern, 1980; Gouteux & Millet, 1984; Gooding, 1988; Vreysen & Van der Vloedt, 1990). Research with other members of the *palpalis* complex are limited to the initial experiments of Vanderplank (1948), who demonstrated the willingness to mate between *Glossina palpalis palpalis* and *Glossina fuscipes fuscipes* and *Glossina fuscipes fuscipes* and *Glossina fuscipes martinii* (*Gfm*) under laboratory conditions. The cross breeding experiments between *Gpp* and *Gff,* presented here, revealed (1) no difference in mating response between the intra- and interspecific crosses, (2) *Gff* males were equally successful in inseminating females of both species but (3) *Gpp* males were more successful in the intraspecific cross than in the interspecific matings. Remarkably, in the experiments conducted by Vanderplank (1948), both *Gpp* and *Gff* males inseminated more females in the interspecific crosses than in matings with females of their own kin. Moreover, although our data indicate semi sterility or reduced fertility in both interspecific crosses, the fertility levels in our experiments were higher than the ones observed by Vanderplank (1948). This is most likely associated with improved rearing conditions in the Seibersdorf laboratories and better adaptation of the flies to the artificial environment. In the cross (*Gff* x *Gpp*), 72 % of the *Gff* females died in the days following the mating. This high mortality was procured by the piercing of the abdomen of the female fly by the superior claspers of the male *Gpp* exacerbated by subsequent bacterial infections of the gut system as evidenced by high 'blood mortality'. This phenomenon is not confined to crosses between *Gpp* and *Gff* and is even more dramatic when a male *Gfm* is mated with a female *Gff* i.e. the extremely large claspers of *Gfm* do not only pierce the gut of the *Gff* females but occasionally, the dorsal side of the female fly is protruded (Vanderplank, 1948). The damage inflicted on the females' abdomen is related to the slightly different morphology of the superior claspers of the different subspecies. The free tooth of the superior claspers of male *Gff* is equipped with a protective membrane which is absent in *Gpp.* These small anatomical differences of the claspers also generate different mating scars as is described in the next chapter. Our data indicate reduced viability of offspring produced by the (*Gpp* x *Gff*) cross (60.5% emergence) but not in the reciprocal cross. These data are again in contrast with the observations of Vanderplank (1948), who found reduced viability in all produced offspring (emergence rate of 33%).

Cross breeding experiments of this kind are usually carried out in relatively small cages and flies of different subspecies, confined to these small areas, might be induced to a mating behaviour not exhibited

under normal natural conditions. Therefore, in an attempt to simulate a more natural situation, an experiment was set up in a large laboratory cage (200 x 180 x 20 cm) where males had to trace the female flies. Again, no indication of assortative mating was found: on the contrary, in both experiments, more matings occurred with males of the other subspecies.

The aim of this study was limited to assess fertility and receptivity of the interspecific crosses. It was not possible to extend this study and assess the fertility of male (no male offspring resulting from the *(Gpp x Gff)* cross was obtained) and female hybrids in back crosses with the parental lines. These studies are currently being conducted at the Entomology Unit of the Seibersdorf Laboratory (Feldmann, pers. comm.). Van der Vloedt (pers. comm.) crossed some of the F_1 progeny back with the parental lines on a limited scale. Interestingly, his data revealed no hybrid sterility in males resulting from the cross *(Gpp x Gff).* Only 2 males were obtained from the reciprocal cross (*Gff x Gpp)* which were mated with 4 females. Females did not produce any offspring and their spermathecae were empty, but this apparent sterility of these male (*Gff x Gpp)* hybrids needs to be confirmed by more quantitative data.

The results of our breeding tests, including those carried out in the large laboratory cages, strongly suggest the feasibility of releasing *Gpp* males in a *Gff* habitat with the majority of the *Gff* females being killed as a result of the mating. Those females not killed in the mating process, will only produce limited offspring. Releasing *Gff* males in a *Gpp* habitat will likewise result in a reduction of the native fly population due to the semi sterility of the crosses. However, there will be no "bonus" effect due to the absence of male hybrid sterility and the extreme sex ratio distortion in favour of (semi fertile?) females. The option of releasing *Gff* males sterilised with a dose of gamma rays in a *palpalis* habitat could overcome this problem. No direct experiments were carried out to assess the effects of radiation on the mating behaviour of the two species. In similar experiments Vreysen & Van der Vloedt (1992) have demonstrated that a sterilising radiation treatment did not alter the mating behaviour of *Gpp* and *Gpg.* The release of gamma sterilised males of one species in the habitat of the other will also overcome a potential major political obstacle i.e. possible restrictions imposed by African governments to import and release fertile species not present naturally in the country.

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Chapter 11

ANALYSIS OF THE MATING SCAR PATTERN OF *Glossina palpalis palpalis* (Robineau-Desvoidy) AND *Glossina fuscipes fuscipes* Newstead (Diptera: Glossinidae)

Abstract

An analysis was made of the mating scar pattern of female *Glossina palpalis palpalis* Robineau-Desvoidy and *Glossina fuscipes fuscipes* Newstead. Measurements on fifty permanent preparations of the mating scars of females reared in the laboratory revealed significant differences in the length, width and in the distance between the centres of the mating scars of the two species. Plotting the distance between the centres of the two mating scars against the ratio width/length resulted in a 93% separation of the two species.

It is proposed that this technique could be used during field surveys to expose possible cross breeding in nature or as a tool in the entomological evaluation of a tsetse eradication campaign where one species is released in the habitat of the other.

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INTRODUCTION

Glossina palpalis palpalis Robineau-Desvoidy (*Gpp*) inhabits the riverine forest galleries of the humid savannah and forest eco-systems in eastern West Africa (Challier *et al.*, 1983). The habitat of *Glossina fuscipes fuscipes* Newstead (*Gff*) coincides with the tropical rain forest of central Africa and is confined to river systems draining into the Atlantic Ocean and the Mediterranean Sea (Ford, 1970). The two species hybridise readily in the laboratory (Vanderplank, 1948; chapter 10) but the existence of hybrids in nature has never been reported despite the fact that islands of *Gpp* occur within the *Gff* area and vice versa (Ford, 1970) in the southern part of Cameroon, Central African Republic and in Zaire (Machado, 1954).

Both species belong to the *palpalis* group, and consequently, the female fly bears on the 6 th tergite mating scars which are the result of the contact of the superior claspers during mating (Squire, 1951). Small morphological differences have been described between the superior claspers of the two species (Machado, 1954), resulting in a slightly different mating scar pattern. This study was carried out to quantify biometrically the mating scar pattern of the two species.

MATERIAL AND METHODS

The flies used for this study were derived from the stock colony of *Gpp* (Nigeria) and *Gff* (Central African Republic) maintained at the Entomology Unit of the IAEA's laboratories in Seibersdorf, Austria. Both colonies are maintained on a membrane feeding system since 1981 and 1986 respectively (Van der Vloedt *et al.*, 1987; Wetzel & Luger, 1978). Fifty permanent preparations were made of the mating scars of females of both species. The tergites of the colony females were separated from the rest of the abdomen, macerated for 5 to 10 minutes in 10% potassium hydroxide and then transferred to 100% acetic acid for 5 minutes. The tergites bearing the mating scars were mounted in Euparal. Measurements of the maximum width and length of the 2 scars of each specimen were made under a Leitz compound microscope at x 40 magnification. For each specimen, the average values of the left and right scar were used in the analysis. The distance between the centres of the mating scars was calculated by subtracting half the width of the left and half the width of the right scar from the distance between the outside borders of the left and right scar. Student's *t*-test was used to compare the means of the length and width of the mating scars and

Table 1. Dimensions of the mating scars of *Glossina palpalis palpalis* and *Glossina fuscipes fuscipes*

Species	Females no.	Dimensions (µm) of mating scars (± SD)		Distance (µm) between the center of the left and right mating scar (± SD)(*)
		Length (*)	Width (*)	
G.p.palpalis	50	296.8 ± 78.8 a	262.6 ± 69.6 a	1137.6 ± 121.2 a
G.f.fuscipes	50	487.3 ± 145.6 b	205.1 ± 46.3 b	899.6 ± 119.2 b

(*) Data in 1 column followed by a common letter are not significantly different (*t* test, $p > 0.05$)

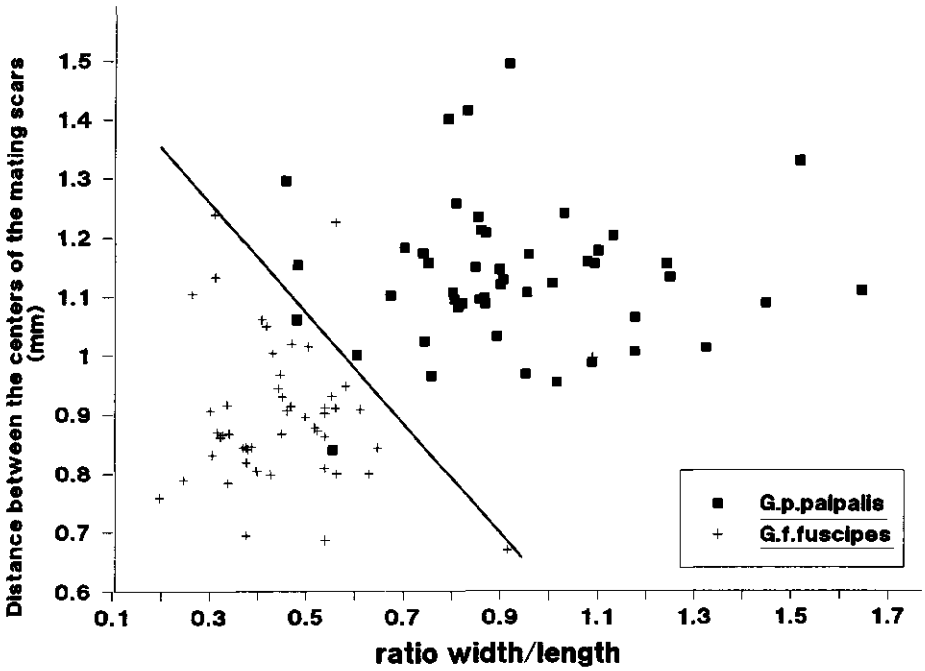

Fig. 1 The ratio width/length of the mating scars plotted against the distance between the centres of the mating scars of *G. p. palpalis* and *G. f. fuscipes.*

the distance between the centres of the mating scars of the two samples. Discriminant analysis (Statgraphics 4.0) was used to discriminate between the two species based upon the variable distance between the centres of the mating scars and the ratio width/length of the mating scars.

RESULTS

Table 1 presents the results of the measurements of the mating scars of the two species. Both the length and the width of the mating scars were significantly different for the two species i.e. an average length of 296.8 µm ± 78.8 µm and 487.3 µm ± 145.6 µm ($p < 0.001$) and an average width of 262.6 µm ± 69.6 µm and 205.1 µm ± 46.3 µm ($p < 0.001$) were measured for *Gpp* and *Gff* respectively. The mating scars of *Gff* were not only longer and more narrow than the one's from the other species, but the two dots were situated closer to the median axis of the abdomen (distance between the centre of the two mating scars was on average 1,137.6 µm ± 121.2 µm and 899.6 µm ± 119.2 µm ($p < 0.001$) for *Gpp* and *Gff* respectively).

Fig. 1 presents a plot of the distance between the centres of the two mating scars against the ratio width to length of the mating scars. Discriminant analysis revealed an almost complete (93%) separation of the two species with only 7 females not clearly classified as either *Gpp* or *Gff*.

DISCUSSION

Squire's (1951) observation of melanisation of the cuticle on the female's 6 th tergite after mating was of the most importance to tsetse workers. Examination of the dark irregular spots on the female's abdomen allowed distinction between mated and unmated females without dissection. The scars are caused by friction of the male 's superior claspers during the mating act. During the process, the protective waxy layer of the surface of the skin most likely is removed resulting in oxidation of the poly-phenols in the epidermis (Squire, 1951). The appearance of mating scars after mating persists throughout the life of the female fly and is restricted to the members of the *palpalis* group. No mating scars are observed after copulation on females of the *morsitans* and *fusca* group owing to the character of the mating scars.

The superior claspers of males belonging to the *palpalis* group are connected with a medially indented membrane. Whereas the general morphological appearance of the two species *Gpp* and *Gff* is fairly identical, the morphology of the male's genital armature is slightly different. The free tooth of the superior claspers in *Gff* bear an obvious median protection which is absent in *Gpp* (Machado, 1954). In this study, it was shown that these small morphological differences in the structure of the superior claspers influence significantly the sites and type of melanisation on the female's abdomen. Whereas *Gpp* males cause 'spot like' mating scars (almost as long as wide), *Gff* males cause more elongated dots closer to the median axis of the abdomen. The morpho-metrical characterisation of the mating scar pattern of the two species based upon the distance between the centres of the mating scars and the ratio width to length described in this paper could have a dual practical purpose:

(1) the technique could provide a valuable tool in tsetse surveys carried out in those geographical areas where both species meet or co-exist. Although both species occur in similar biotopes, have identical ecological requirements and overlap in some areas, no specimen with morphological intermediate characteristics has so far been reported (Machado, 1954). This is not surprising in view of the fact that only hybrids resulting from the cross female *Gpp* X male *Gff* can potentially exist i.e. mating between a female *Gff* and a male *Gpp* results invariably in the killing of the majority of the females due to piercing of the female's abdomen (Vanderplank, 1948). Analysis of the mating scars of *Gpp* females could expose potential breeding with *Gff* males.

(2) the willingness to mate under laboratory conditions between *Gpp* and *Gff* was demonstrated by Vanderplank (1948). In addition, exposing *Gpp* females and *Gff* females to equal densities of sexually mature males of the two species in large laboratory cages (200 x 180 x 20 cm), revealed at random mating (Chapter 10). These data are in accordance with the observations made with the two subspecies *G.p. palpalis* and *G p.. gambiensis* (Vreysen & Van der Vloedt, 1990). This apparent absence of preferential mating between closely related species or subspecies of the *palpalis* group could be used as a genetic control method by releasing irradiated males of one species in the habitat of the other (Vreysen & Van der Vloedt, 1990). In the case gamma sterilised *Gpp* males were released in a *Gff* habitat, control would be achieved by the killing of the female *Gff* population as a result of the cross-mating (Vanderplank, 1948) and due to induced sterility in those *Gff* females

not killed in the mating act. In the reverse situation, all cross matings would result in sterile *Gpp* females, caused by embryonic arrest in the developing embryo. Monitoring of these release programmes can rely on biological evaluation methods which are based upon imbalances in the reproductive system of the captured female flies (Van der Vloedt & Barnor, 1984). However, in some reproductive phases (females who are nulliparous, have a recently ovulated egg *in utero*, or have an empty uterus possibly due to a recent larviposition) the mating with a sterile male is obscured. Analysis of the mating scar pattern of trapped *Gpp* and *Gff* females could provide accurate information on the rate of cross-mating and would supplement the traditionally used biological evaluation methods (Van der Vloedt & Barnor, 1984).

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Part 3 The Sterile Insect Technique in an integrated approach for the eradication of *Glossina austeni* on the island of Unguja (Zanzibar)

Chapter 12

THE HISTORY OF TSETSE AND TRYPANOSOMIASIS RESEARCH ON UNGUJA ISLAND (Zanzibar)

General background information of Unguja island

Zanzibar comprises the islands of Unguja and Pemba both situated east of mainland Tanzania. Unguja island covers an area of ± 1,600 km^2 with a maximum north - south distance of 80 km and 35 km in an east - west direction (Fig. 1). In contrast to Pemba island, Unguja was connected with Tanzania mainland during the Pleistocene. Now, it is separated from the East African coast by the 35 km wide Zanzibar channel. With the highest elevation less than 150 m, the island has an equatorial and monsoonal climate characterised by a relative constant temperature, high humidity and an average annual rainfall of 1,600 mm. The rainfall pattern is bi-modal with the main rainy season from March to May and a lesser rainy period in November - December. No month is however without rain. Two dry seasons can be distinguished i.e. the cold dry season between June and October and the period between January and March which has the highest temperatures. The average temperature is 27 °C with a mean maximum of 29 °C and a mean minimum of 25 °C. The north-western part of the island has deep, rich soils, derived from Miocene non-calcareous sediments whereas in the east and southern areas, the soils are shallow and derived from recently uplifted reefal limestone (Turner, 1984). Demographically, the island is densely populated with more than 200 inhabitants per km^2. The current population of 400,000 grows with 3% annually. The highest concentration of people are found in Zanzibar town and in the north and western parts of the island. The population pressure on natural resources is high and consequently, most of the original vegetation has been removed and replaced by cultivations: coconut, mango, cloves, banana and rice fields. The majority of the cultivated land is situated on the western side of the island where the soils are rich and adequately watered. Until recently, the economy was principally supported by the export of cloves before the international clove market collapsed due to tense competition from Madagascar and the Far East. As a response to the reduced clove prices, new trends of development are becoming apparent with the appearance of sugarcane, rice plantations and other cash crops such as coffee, cocoa, citrus and rubber. The World Food Programme sponsored

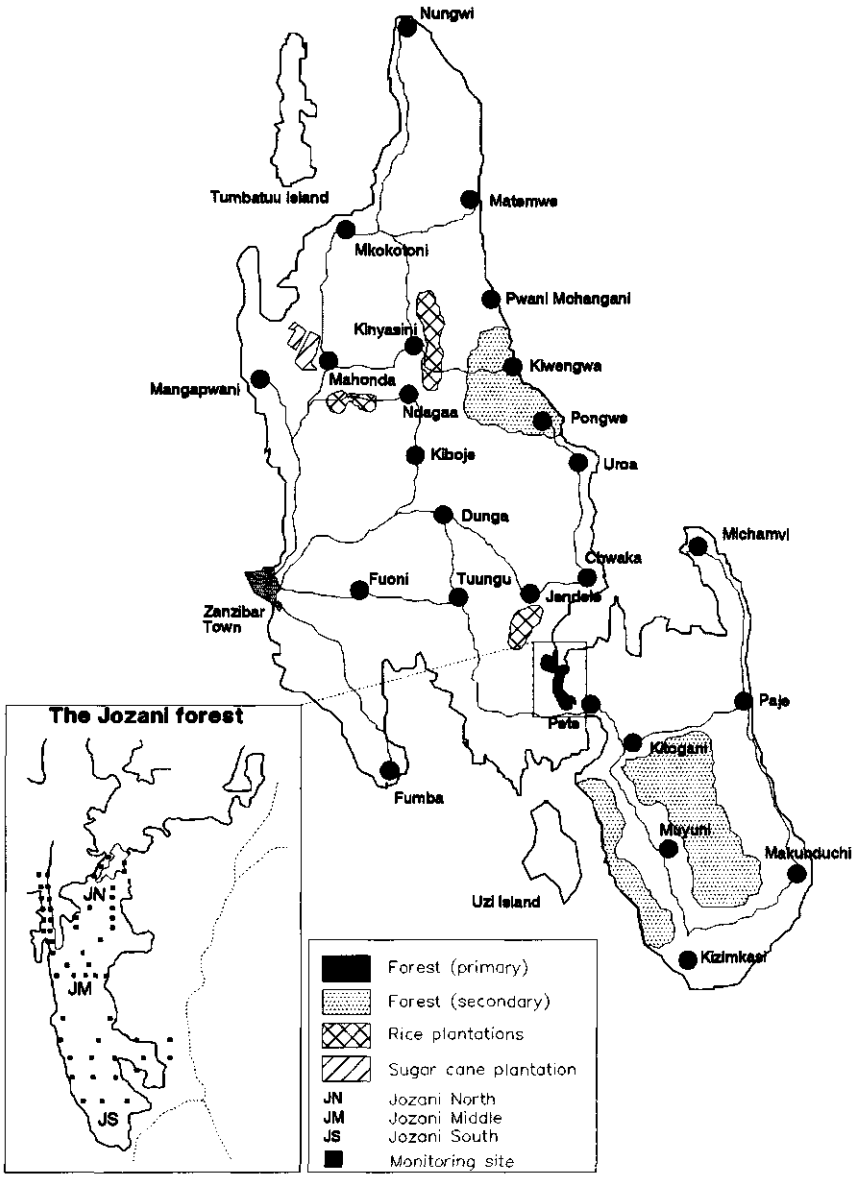

Fig. 1 An outline of the island of Unguja indicating the main forested areas.

livestock census of 1993 revealed 45,750 head of cattle (65,943 on Pemba), 26,472 goats (18,643 on Pemba), 375 sheep (265 on Pemba) and 494 (700 on Pemba) donkeys on Unguja island. Cattle, mostly East African Zebu crossed with Boran and Sahiwal breeds, are usually owned by small holders (1-4 head of cattle) who leave the cattle grazing on native grasses under clove, coconut plantations and on fallow land between cassava and other food crops. The farmers produce their crops by shifting cultivation and consequently, bush regeneration is encouraged to fertilise the soil. This practice however creates ideal habitats for *G. austeni* e.g. the Mangapwani area (Schönefeld, 1988). No attempts are made to manage the pastures, with overstocking and weeds expansion as a result.

The only remaining primary forest is the Jozani forest reserve (Fig. 1) (freshwater swamp forest, high forest and evergreen thickets) where the main tree species are *Calophyllum inophyllum, Elais guineensis* (oil palm) and *Pandanus rabaiensis* (Screw Palm). Secondary forest (coral rag thickets) can be found in the southern (coastal and central Muyuni forest) and middle eastern part of the island (Kiwengwa forest). Extensive mangrove eco-systems still exist north of Jozani, Ukongoroni, Charawe, between Unguja Ukuu and Uzi island and in the north of Mkokotoni. Like all other original vegetation, it is under heavy pressure due to logging and extensive pole cutting mainly for ceiling construction. The fauna is poor due to long intense human activity and clearing of most of the natural vegetation. The island has few endemics on species level but considerable number on subspecies level (Müller & Nagel, 1994).

Tsetse and trypanosomiasis on Unguja

The disease trypanosomiasis was first reported in 1908 on the island of Unguja, but circumstantial evidence is reported as far back as 1880 (Johns, 1952). No human sleeping sickness occurs on the island. A direct association seems to exist between the abolishment of slavery in 1897 and the spreading of the tsetse fly from their permanent habitats in the southern thickets to the middle and western parts of the island. Arab owned plantations, which were previously maintained and cleaned by free slave labour, were neglected and left to fallow, creating suitable tsetse habitat. The elusiveness of the fly is illustrated by its late discovery on the island: a fly boy (brought over from the mainland?) caught the first tsetse fly in 1945 in *Leucaena glauca* thickets in Chukwani. During a first island wide tsetse survey, carried out between 1948 and 1951, it was revealed that (1) *G. austeni* was the only species present, (2) the fly was widespread over the island and (3) was concentrated in the coral thickets of the eastern part of the island (Johns, 1952). During the same period,

more than 8,000 head of cattle were screened for the disease showing an overall infection rate of 17%, being predominantly *T. congolense* and to a lesser extent *T. vivax.* The incidence of trypanosomiasis peaked during the rainy season and it was therefore assumed that fly distribution was seasonally influenced i.e. retreating during the dry season in the permanent foci and more dispersing during the rains. Johns (1952) proposed reclamation of the western area of the island by bush clearing but no attempts were made.

In the seventies, the island received numerous missions of tsetse experts (C.S. Tarimo of the Tropical Pesticide Research Institute in Arusha, L. Otieno & Chaudhury of ICIPE, in Nairobi, A.M. Jordan of the Tsetse Research Laboratory in Bristol, U.K. and an USAID mission assessing the feasibility of using SIT on the island), all stressing the urgent need for a thorough tsetse survey covering the entire island. More serious attempts to assess the tsetse and disease problem were made in the eighties. Mr. J.G. LeRoux of the Food and Agricultural Organisation reviewed the tsetse situation on the island in 1981 and concluded that (1) the distribution of the fly was limited to Mangapwani and the Jozani forest and that (2) the coral rag thickets of the east and south did not harbour any fly population due to its unfavourable habitat and lack of host animals. Again, an extensive tsetse and trypanosomiasis survey was recommended. During a revisit in 1982, Mr. LeRoux pioneered a first control attempt by positioning 37 black cotton screens, impregnated with 3% solution of dieldrex at an interval of 100 m. The trial was carried out in the Mangapwani area (12-15% trypanosomiasis incidence) but failed due to inefficient monitoring procedures (pre-control monitoring using fly rounds and biconical traps caught 1 fly), theft of the majority of the screens and the early removal of sentinel cattle due to shortage of grazing land.

In 1983, the Government of Tanzania requested assistance to the IAEA for the eradication of *G. austeni* from Unguja by means of the Sterile Insect Technique. A colony was initiated at the Trypanosomiasis and Tsetse Research Institute located in Tanga, Tanzania from pupae collected in the Jozani forest. Flies were originally maintained on goats and rabbits, but the *in vitro* feeding technique was introduced in 1984 (Tarimo *et al.,* 1988). By mid 1990, the colony had reached a size of 40,000 females, producing sufficient excess males to initiate pilot release studies on the island (Vreysen, 1990; Vreysen, 1991).

In the mean time, serious attempts were made to assess the fly distribution and gain base line data on fly ecology. Failing to trap significant numbers of adult flies, Turner (1984) had to rely on pupal searches to assess the fly distribution over the entire island. During

these pupal searches in 64 locations, marked associations were found between the north-south ridges of raised reefal limestone and breeding sites. Pupae were likewise located in dark, loamy soil underneath exposed buttress roots of large trees, under large trees with trunks sharply angled to the ground and under fallen logs. Turner (1984) concluded that (1) the Jozani forest is the largest permanent habitat of *G. austeni* with (2) a more 'fluid' distribution in the eastern parts as a result of the shifting cultivation and fallowing of the vegetation, (3) most of the central middle and western part of the island is tsetse free, with the exception of Mangapwani, due to lack of relic forests and agricultural expansion and (4) seasonal factors do not play a major role in the distribution of the fly. This entomological work was continued during missions and assignments of Hall (1986), Madubunyi (1989) and Hall (1990) who concentrated on the improvements of traps and introduced the sticky panel.

In 1987, a pilot trial was initiated at Mangapwani under the initiative of FAO, to test the efficacy of the pour-on method (livestock treatment with a residual synthetic pyrethroid) for the control/eradication of *G austeni* (Schönefeld, 1988). Monitoring of the fly population by means of the 3 Dimensional sticky panel revealed an average pre-control apparent density (A.D.) of 1 fly/panel/day (0.14-3.66) whereas a pre-control trypanosomiasis incidence of 46% was found in the cattle using the Hematocrite Centrifuge Technique (HCT). Application of the insecticide on 700 head of cattle, 200 goats and a few donkeys in 5 cycles spaced by 15 days, resulted in a drop of apparent fly density to zero within 37 days. None of the sentinel animals became re-infected. This highly successful trial culminated in an island wide eradication effort using the pour-on technique in areas with abundant livestock and Insecticide Impregnated Screens (IIS) in those areas where livestock was absent (Höreth-Böntgen, 1992). Although a good level of control was achieved, the programme failed to eradicate the species from the entire island (FAO/UNDP, 1993; FAO/UNDP, 1994)

Survey techniques

Bait oxen.

Bait oxen are traditionally considered to be the best means of trapping *G austeni* with fly boys, vehicles and traps being ineffective. Moggridge (1949) reports that 2 children could catch as many *G. austeni* from an oxen in 2 minutes as a Harris or Swynnerton trap (see Introduction) in 20-50 hours. Oxen fly rounds conducted at Mkwaja ranch (Tanzania) in 1957-58 detected *G. austeni* (Napier Bax, reported by Gates *et al.,* 1983) but fly

rounds conducted in the same area in the seventies by 2 man carrying a black screen (1.0 x 1.3 m.) between them failed to do so (Gates *et al.*, 1983). It is therefore not surprising that bait animals were the preferable survey tools during initial trapping efforts on the island (Hall, 1986; Hall, 1990). Results were however not very encouraging with only 2 flies being trapped during a 1.5 hour trial in the Jozani with 2 cows (although 12 flies were reported as 'seen'). Similar results were obtained with a goat i.e. 3 flies trapped (7 seen) during a 1.8 hour fly round. Later trials with fly boys and bait oxen did however not catch any flies (Mramba *et al.*, 1991). Although maybe more efficient than conventional traps, bait oxen is not a very practical way of surveying *austeni* populations and proved to be completely impossible in dense forest habitats like the swamp forest of Jozani or the coral rag thickets of the eastern part of the island. Goats might offer more potential than cows but the practice has never been implemented on an operational basis.

The biconical and other tsetse traps

Initial attempts to trap adult flies using the biconical trap (Challier & Laveissière, 1973) failed completely and its inefficiency for catching *G austeni* became very rapidly clear: catches fluctuated between zero (Hall, 1986) to 0.2 flies/trap/day (baited with acetone, methyl ethyl ketone and methyl vinyl ketone (Turner, 1984)) and 0.88 flies/trap/day (Madubunyi, 1989). The latter samples however included the flies caught on the sticky 'ant barrier' part of the supporting pole (92.8% of the catch). The corrected catch in the non-return cage was 0.06 flies/trap/day. Modifying the biconical traps by lowering the trap and increasing the dimensions of the entrance holes did not increase the catch rate (Hall, 1986). Biconical traps located in other areas (Mangapwani, Muungoni, Kisauni) failed likewise to catch any flies (Turner, 1984). Similar results were obtained by Takken (1984) who reports catches of 0.05-0.12 *G. austeni* flies per biconical trap per day in Mozambique. On many occasions, he observed that the fly had entered the trap but would not proceed towards the non-return cage. Many flies were found sitting on the inferior baffles inside the trap. Trials with the Lancien trap (another trap designed for *palpalis* species) were equally unsuccessful (Höreth-Böntgen, reported by Hall, 1990).

These unsuccessful attempts with the biconical trap, prompted researchers to conduct trials with traps designed for *morsitans* species i.e. the F3 and Epsilon trap. No improvements were however obtained with catches between 0.1 (Hall, 1986) and 1.0 fly/trap/day (Madubunyi, 1990).

Trials with the canopy trap (used for trapping Tabanids that, like *G austeni,* alight on legs and the belly of animals (Hall, 1990)), were equally

unsuccessful. The highest catches with conventional tsetse traps were recently reported by Owaga (pers. comm.) who trapped 1 to 11 *G. austeni*/trap/day with blue-black and lila-black pyramidal traps.

Sticky panels

The concept of using sticky panels to trap *G. austeni* was introduced by Hall (1986) who tested black and white sticky legpanels in the Jozani forest. Up to 1.2 flies/panel/day were caught with the white panel but no flies were trapped with the black version. The unattractiveness of black for *G. austeni* was a confirmation of a small experiment carried out by Turner (1984) using a black screen made sticky with isopolybutylene. Contradictory to the observations of Madubunyi and our data (Chapter 13), Hall (1986) found only 27% (4/15) of the flies landing on the legs with the majority of the flies sticking on the panel body (0.5 m above ground). This is also in contrast with Hall's own observations with bait oxen: 11 out of 13 flies were recorded on the legs and only 2 out of 13 on the lower abdomen of the bait oxen. Likewise, all flies recorded on the goat were seen on the legs.

During efforts to elucidate the trap oriented behaviour of *G. austeni* electrical nets were introduced. During those trials, 92% of the flies were trapped on the nets covering the targets and only 7% on the netting to the side of the target indicating that *G. austeni* (contrary to *G. pallidipes* and *G. morsitans morsitans*) do not circle a target but alight immediately on it (Hall, 1986).

Following recommendations by Hall (1986), Schönefeld (1988) constructed a 3-dimensional sticky panel (3D) (Fig.1,chapter 13) consisting of 3 arms set at 120° to one another. Suspended from a branch it caught on average 1.02 flies/trap/day in the Mangapwani area and was used routinely for monitoring activities of the UNDP/FAO Animal Disease Control Project (Höreth-Böntgen,1992). Madubunyi (1989,1990) developed the Chuka trap (Fig. 1, chapter 13) made out of plywood board (70 x 61 cm), supported by a metal frame. Friction however prevented free rotation. Madubunyi concluded that the CT was more efficient for trapping *G. austeni* than the 3D (in his trials the 3D was however modified from its original design) and he suggested that the blue version of the CT was more effective than white (but no significant differences in trap catches were observed). His experiments remain therefore somewhat inconclusive. Another, cross shaped, free rotating (XT) sticky trap was tested but only 6 flies were trapped in 2 days with 2 traps (Hall, 1990). This version of the sticky panel proved to be the most efficient for trapping *G. austeni* in

Zululand (South Africa) and is currently being used for survey purposes (Dr. Nevill, pers. comm.).

A free rotating, suspended version of the CT (Monopanel (MP)) (see chapter 13) was used for all entomological monitoring activities of the IAEA supported SIT project (Vreysen *et al.,* 1992). The white MP, made sticky with polyisobutylene.LMW (low molecular weight) caught up to 10.0 females and 12.8 males per panel per day in some of the monitoring sites in the northern part of the Jozani forest (Vreysen, 1992) refuting the long believed assumption of very low fly densities. This panel was replaced as of August '94 by the Blue-White Legpanel (chapter 13).

Response to odours.

Bursell (1984) showed that *G. austeni* had a strong kinetic response to acetone and carbon dioxide in the laboratory which was confirmed in Mozambique by Takken (1984) and on Unguja island by Hall (1986). Although traps were baited with olfactory odours in most of the experiments of Turner (1984) and Hall (1986), no systematic research on the effects of each of the odours was reported. Sticky leg panels baited with buffalo and pig urine (Hafidh, 1987) and biconical traps baited with buffalo urine, pig urine, acetone and octenol did not increase the catch rate (Mramba *et al.,* 1991). However, caution is required in the interpretation of these data as the experiments were conducted without rotation, were not carried out simultaneously and/or details of the experimental set-up and statistical analysis are lacking. Recently, Owaga (pers. comm.) reported increased catch rates with buffalo urine and dry ice (2 to 9 times) with biconical, pyramidal and Ng2b traps on the Kenya coast. The effect of cattle urine was inconsistent.

Ecology of the fly

Very little information is available on the ecology and behaviour of *G. austeni,* which is not surprising in view of the difficulties experienced in trapping *G. austeni.* Both on the African continent and on Unguja island, *G. austeni* is a very elusive and discreet fly, completely silent in flight. It was assumed that the fly was mainly active at night (Moggridge, 1948) but these observations were recently contradicted by Owaga *et al.* (1993) who observed a V shaped activity pattern of *G. austeni* on the Kenya coast, with significant peaks between 09.00 - 10.00 h. and 14.00 - 17.00 h. On Unguja island, the fly was likewise mainly active between 11.00 - 13.00 h. and between 15.00 - 17.00 h. (n = 63) during the period June - July (end rainy season, begin dry cold season). When the temperature increased (0ctober -

November), the main activity shifted towards the end of the day, occurring between 16.00 and 18.00 h. although some activity was observed throughout the day (n = 24) (Mramba *et al.*, 1991). Likewise, at the end of the hot - dry season (March), 80% of males were sampled in the afternoon and late evening (22% between 14.00 and 15.00 h., 17.1% between 15.00 and 16.00 h., 25.7% between 16.00 and 17.00 h. and 14.2% between 17.00 and 18.00 h.). Male flies were very little active after 18.00 h. and before 7.00 h. (8.6%) and in the morning between 7.00 h. and 14.00 h. (Vreysen, 1993). Activity of the females (n=15) was scattered throughout the day, but the data remain inconclusive due to the small sample size.

Bush pigs (46.6%) are the main source of food for *G. austeni* with cattle (14/5%) and duiker (7.1%) contributing substantially to the diet (data from 408 blood samples) (Moloo, 1993). A survey conducted in 1958 - 61 on Unguja island confirmed these data with 50 - 80% of the bloodmeals being derived from bush pigs (Zanzibar Archives, quoted by Bouvry-Stratford, 1986). The fly is not attracted to man and rarely feeds on him. Despite high initial densities of *G. austeni* in the Jozani forest (in some areas up to 50 male flies were trapped on a single panel in 24 hours) none of the staff ever experienced a feeding attempt of the fly. This last observation is contradictory to the observations of Moggridge (1948), who reports that 120 *G. austeni* were caught per hour from a party of men. Likewise, Nash *et al.* (1966) states that Zanzibar collected *G. austeni* refused to feed on domestic pigs, but 'would feed avidly if transferred to the human arm'. This enigmatic feeding behaviour is once more corroborated by the fact that *G. austeni* is the only fly colonised at the IAEA's laboratory at Seibersdorf that thrives on cow blood without pig blood being added.

These findings on distribution and ecology of *G. austeni* on Unguja island were the only information available to serve as a basis for the Sterile Insect Technique eradication programme on Unguja.

Chapter 13

EVALUATION OF STICKY PANELS TO MONITOR *Glossina austeni* Newstead (Diptera: Glossinidae) POPULATIONS ON UNGUJA ISLAND (ZANZIBAR)

Abstract

Monitoring of *Glossina austeni* populations in the forested areas of the island of Unguja (Zanzibar) has since November 1990 routinely been carried out with the sticky panel as commonly used tsetse traps (Biconical, Epsilon, F3) have proven to be unsuccessful in catching *G. austeni*. Initial studies on the catching ability of various types of sticky panels for *G. austeni*, have indicated that the monopanel was as efficient in catching flies as the 3-Dimensional version and the smaller legpanel. No significant differences in catch rate and sex ratio were observed with monopanels in various colours and colour combinations. Legpanels coloured white on one panel side and blue on the other side caught significantly more flies as compared with other colour combinations, but female flies were under sampled (31.7%). The type of sticky material applied on the panel influenced significantly the catch rate and female ratio.

During long term trapping with the 'baby blue' and white monopanel, females were under sampled (37.7 - 46%) except when Polyisobutylene.LMW was used as sticky material. Analysis of the age composition of the sampled *G. austeni* females revealed that tenerals and nulliparous were well represented (11 - 24%). More than 20% of the trapped females were older flies i.e. females with 4 or more ovulations but this percentage dropped to 10% when Tanglefoot was used as sticky material.

It is concluded that the sticky legpanel, coloured sky blue on one side and white on the other side is the most efficient device for monitoring *G. austeni* populations. The non-setting adhesive of choice is Temoocid.

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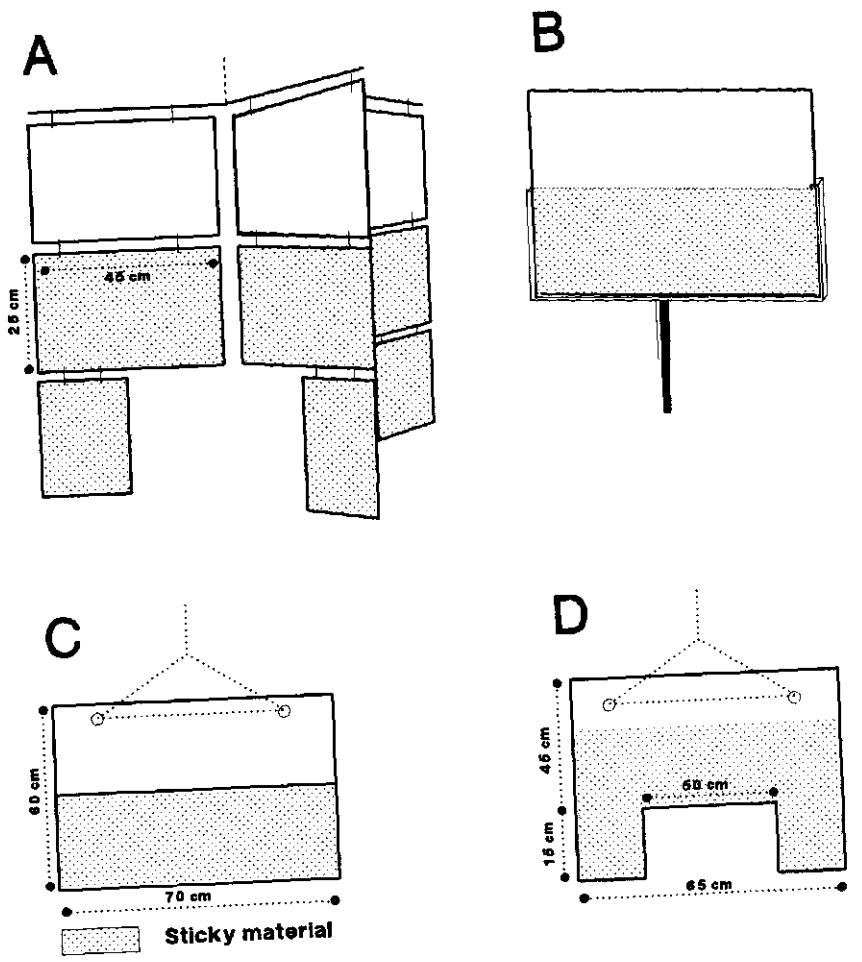


Fig. 1 Diagrams of sticky panels. A: 3-Dimensional Panel (3D)(Schönefeld, 1988), B: Chuka trap (Madubunyi, 1990), C: Free rotating monopanel (MP) and D: Free rotating Legpanel (LP).

INTRODUCTION

Glossina austeni is the only tsetse species present on the island of Unguja (Zanzibar) and solely responsible for the cyclical transmission of animal trypanosomiasis (Johns, 1951). Studies to obtain base-line data on ecology and behaviour of *G. austeni* have always been hampered by the elusive behaviour of the fly and the low catches with standard tsetse traps. During an island wide survey, Turner (1984) had to rely exclusively on pupal searching for assessing its distribution and apparent densities. Further studies using biconical, F3 and Epsilon traps (see 4.3.1. of Chapter 1) gave limited results due to insufficient fly numbers being caught (Turner, 1984; Hall, 1986, 1990; Madubunyi, 1990). Since Hall (1986) initiated tests with panels made sticky with glue, various versions have been constructed and used for surveys and monitoring purposes (Fig.1) (Schönefeld, 1988; Madubunyi, 1990; Höreth-Böntgen, 1992). A suspended, free rotating version of the Chuka trap (Madubunyi, 1990) , has been used since November 1990 to monitor fly population dynamics during control operations in the Jozani forest (Vreysen, 1992 a,b). This paper presents data on performance of the sticky panel in terms of sample composition and its catch rate for *G austeni* in relation to panel shape, colour and sticky material.

MATERIAL AND METHODS

Working area, tsetse sampling and monitoring

All entomological work was carried out in the Jozani Forest Reserve from November 1990 to November 1992. Situated at ± 35 km from Zanzibar town between 6°15' - 6°16'S and 39°24' - 39°25' E, it comprises an area of 10 km^2 and is the only remaining true primary forest on Unguja island.

A modified version of the Chuka trap (Madubunyi, 1990) i.e. monopanel (MP) was used for the long term sampling. They were constructed from 4 mm thick plywood or white PVC material and painted with locally purchased water resistant enamel paint. Panels were made sticky by applying a thin layer of sticky material (Tanglefoot, Polyisobutylene.LMW (low molecular weight), Polyisobutylene.HMW (high molecular weight) and Temoocid) to the bottom half of the panel. All panels were suspended with a rope from an overhanging branch permitting free rotation and were positioned for 2 - 5 days in reference monitoring sites (Vreysen, 1992a). Panels were

checked daily, all trapped flies removed and females dissected for ovarian ageing (Challier, 1965).

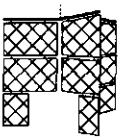
Experimental designs for assessment of panel - glue efficiency

The 3-Dimensional sticky panels (3D) were constructed from fibreglass panels of 0.25 x 0.45 m each, as originally designed by Schönefeld (1988)(Fig. 1). A smaller legpanel (LP) was constructed from 4 mm thick plywood or PVC based upon the original design of Hall (1986) with a body of 0.65 x 0.45 m and 2 legs of dimensions 0.15 x 0.15 m Wooden or PVC monopanels (MP) of dimensions 0.7 x 0.6 m were used as described before (Vreysen,1992a). All panels were equally coloured (baby blue) and coated with Isopolybuthylene.HMW during experiments assessing the effects of panel shape on the catch efficiency. The effect of baby blue (J.L. Morison Son & Jones Ltd., Dar es Salaam, Tanzania), Sky Blue (18 E 53), Light blue (20 E 51) and White (full gloss) (Berger Paints Emirates Ltd., Sharjah, U.A.E.) was tested both with MP and LP in following colour combinations:

	Side A	Side B
SBMP	Sky blue	Sky blue
BBMP	Baby blue	Baby blue
WMP	White	White
BB-WMP	Baby blue/White	Baby blue/White
SB-WMP	Sky blue/White	Sky blue/White
SB/WMP	Sky blue	White
SBLP	Sky blue	Sky blue
LBLP	Light blue	Light blue
SB/LBLP	Sky blue	Light blue
SB-LBLP	Light blue(top)/ Sky blue (bottom)	Sky blue (top)/ Light blue (bottom)
WBLP	White	Sky blue/Light blue
W-BLP	White (left)/ Sky blue (right)	White (left)/ Light blue (right)
BBLP	Baby blue	Baby blue

The effect of different types of sticky material on the catching ability for *G. austeni* was evaluated. Wooden 'baby blue' monopanels were made sticky on each side with one experimental non-setting adhesive applied on the left half, the other non-setting adhesive on the right half. In this

Table 1 Average daily catch and female ratio of 3D, MP and LP and of MP in various colour combinations in the Jozani forest.

	Trap type	Average daily catch	SD	F-Ratio (sample size)	Females %
Exper. 1		3.3	1.8	7.2 ns (248)	51.0
		9.6	6.7		45.8
		3.7	2.5		50.9
Exper. 2		6.2	4.8	0.7 ns (113)	45.2
		2.8	0.5		42.9
		5.4	5.4		37.0
		3.6	1.8		5.5
		4.6	2.4		39.1
Exper. 3		4.1	2.2	2.0 ns (149)	45.5
		1.8	1.5		35.7
		1.8	0.7		41.4
		1.6	1.2		53.8

ns : non significant

way, catch rate of Polyisobutylene.LMW (Aldrich Chemical Co., Milwaukee, USA) was compared with catch rate of Hyvis 2000 (BP. Chemicals Ltd.), Oecotac A 10 (Oecos Ltd.) and Polyisobutylene.HMW (Aldrich Chemical Co., Milwaukee, USA). The latter was compared with Hyvis 2000 and Temoocid (Kollant SPA, Italy). Whereas Temoocid (liquid) and Tanglefoot (viscous) were applied undiluted, kerosene was added to Hyvis 2000, Oecotac A 10 and Polyisobutylene.LMW before application on the panels. The solid Polyisobutylene.HMW was warmed up in order to obtain a liquid state making dilution with water possible. Small quantities of kerosene were added to the extreme viscous form of sticky material for easy application on the panels.

All experiments were performed in the northern half of the Jozani Forest Reserve and carried out in 3 x 3, 4 x 4, 5 x 5 or 6 x 6 Latin Squares. All panels were checked daily and females dissected for ovarian ageing. Catches were expressed as the average number of flies caught per panel per day and subjected to a log(n + 1) transformation prior to analysis of variance. Differences between treatment means were tested for significance using Duncan's multiple range test.

RESULTS

The effect of panel shape

The average daily catch per panel and female ratio of various trap types and colour combinations are presented in Table 1. No significant differences in catch rate were observed by analysis of variance between 3D, MP and LP. The proportion of females was similar in samples of 3D and LP (51%) and slightly under represented in the MP samples (45.8%, chi square = 0.8, $p > 0.05$). The physiological age distribution of the females sampled with the MP was comparable with the one found on the LP with 23.6 and 30.8% tenerals & nulliparous, 60 and 61.5% females with 1-3 ovulations and 16.4 and 7.7% females with 4 or more ovulations for the MP and LP respectively (Fig. 2). Young females however, were much better represented in the 3D samples (60.9% nulliparous) but the proportion of females with 4 or more ovulations was limited to 4.3% (chi square = 12.3, df= 4, $p < 0.05$).

The effect of colour

In experiments to assess the effect of colour on the catch rate of the MP (Table 1), most flies were caught on the SBMP (average catch of 6.2 ± 4.8 and 4.1 ± 2.2 flies/panel/day in the two successive series

respectively) but the differences were not significantly different. Female ratios varied between 35.7% (BBMP) and 53.8% (SB/WMP) but were not significantly different from a 1:1 ratio (chi square, $p > 0.05$). A completely aberrant female ratio (5.6%) was obtained on the BB-WMP. During observations on the landing positions, the majority of female (80%) and male flies (82%) were found sticking on the bottom area (0 - 15 cm) of the panel.

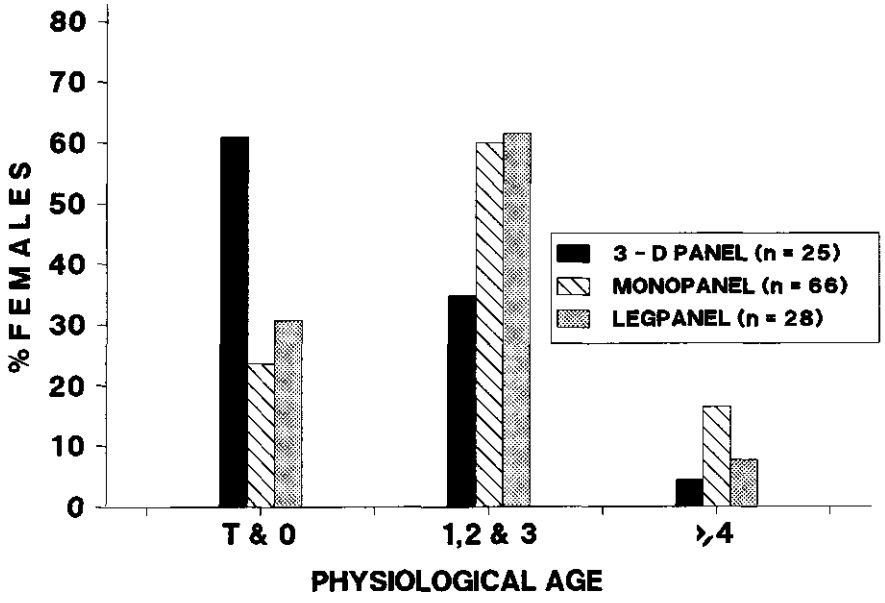
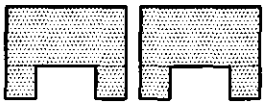
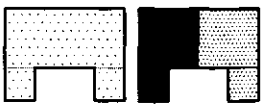
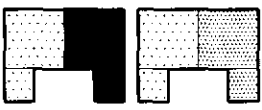


Fig. 2 Frequency distribution of physiological age of female *Glossina austeni* caught on 3 - dimensional sticky panel, monopanel and legpanel in the Jozani forest. (chi square = 12.265, df = 4, $p < 0.05$)

In the trapping experiment using various colour combinations of legpanels, WBLP caught significantly more flies ($p < 0.05$) but only 31.7% were females (chi square = 7.68, $p < 0.001$) (Table 2). 63% of the fly sample of WBLP, were found sticking on the blue side of the panel (chi square = 4.06, $p < 0.05$). With the exception of SB/LBLP and SB-LBLP, all other colour combinations caught significantly more males (chi square, $p < 0.05$).

Table 2. Average daily catch and female ratio of LP in various colour combinations in the Jozani forest.

Panel (Side A / Side B)	Average daily catch	SD	F-Ratio (sample size)	Females %
	3.3 b	2.9	7.3 * (143)	25.0
	3.7 b	4.3		9.1
	1.7 b	1.9		20.0
	3.2 b	1.5		47.4
	10.5 a	6.4		31.7
	1.5 b	1.6		11.1

Average catches followed by the same letter are not significantly different ($p > 0.05$). (ns = non significant, *: $p < 0.05$)

The effect of the sticky material

Table 3 presents the results of the various experiments carried out to assess the effect of the different types of sticky material. The Polyisobutylene.LMW side of the panels caught significantly more flies than the area made sticky with Polyisobutylene.HMW, Hyvis 2000 and

Table 3. Catch rate of 'Baby Blue' monopanels coated with different types of non-setting adhesives. Panels were divided in half A (left) and half B (right) on both sides .

Type of glue on panel		Number of			Flies caught on A half of panel			Flies caught on B half of panel		
half A	half B	catch days/panels/flies			females no.	males no.	% of total	females no.	males no.	% of total
Hyvis 2000	Polyis.LMW	4	5	138	4	10	10.1	62	62	89.9
Oecotac A10	Polyis.LMW	2	3	28	0	6	21.4	16	6	78.6
Polyis.HMW	Polyis.LMW	3	5	200	42	33	37.5	64	61	62.5
Polyis.HMW	Temoocid	6	7	258	13	46	22.9	86	113	77.1
Polyis.HMW	Hyvis 2000	5	4	90	26	37	70.0	4	23	30.0

Oecotac A10 (chi square, $p < 0.01$). Polyisobutylene.HMW caught fewer flies as compared with Temoocid (chi square = 74.8, $p < 0.001$), but caught significantly more than Hyvis 2000 (chi square = 86.09, $p < 0.001$). Males were much better represented in the catches of Hyvis 2000 (chi square = 14.04, $p < 0.001$), Oecotac A10 (chi square = 4.16, $p < 0.05$) and Polyisobutylene.HMW (chi square = 5.86, $p < 0.05$) whereas females and males were caught in equal numbers on the Polyisobutylene.LMW and Temoocid (chi square, $p > 0.05$)..

Long term sampling

Data on age structure, female ratio and variations in the pregnancy stages of females sampled with the BBMP and WMP glued with various types of sticky material are shown in Table 4. Females were significantly under represented in all samples (chi square, $p < 0.01$) except in the catches with BBMP made sticky with Polyisobutylene.LMW (chi square = 0.05, $p > 0.05$). There was a significant association between panel colour/sticky material and the age composition of the female flies (chi square = 83.15, df = 12, $p < 0.001$). A lower proportion of Tenerals and Nulliparous flies were caught with BBMP/Polyisob.LMW and WMP/Temoocid (11.4% - 15.5%) whereas the BBMP/Tanglefoot caught less old females (4 or more ovulations).

There was likewise a significant association between panel colour/sticky material and the reproductive status of the female flies (chi square = 46.01, df = 12, $p < 0.001$). Equal proportions of females with an egg (46 - 49%) and with an immature larva (46 - 51%) *in utero* were caught on the BBMP glued with Polyisobutylene.LMW and HMW and on the WMP made sticky with Temoocid. The BBMP/Tanglefoot combination caught fewer flies with an egg *in utero* and more flies with a first or second instar larva *in utero*. The later pregnancy stages (third instar) were under represented in all samples (from 0.0 to 2.1%).

DISCUSSION

In the intitial studies of Hall (1986) and Madubunyi (1990), significantly more *G. austeni* flies were caught with the 'sky blue' and white sticky panel than with the biconical trap. Our trapping experiments with 'sky blue', white and 'baby blue' MP showed however non-significant differences in sample size. Observations with other tsetse species have clearly demonstrated the superiority of 'royal blue' (phtalogen) as a colour in traps and screens. Its high reflectivity in mid blue (460 nm) combined with its low reflectivity in ultra violet and

Table 4. Age structure and uterus content of female *Glossina austeni* sampled with sticky monopanels in Jozani forest

Panel colour	Sticky material	Sample size	Females	Tenerals & nulliparous	Parous females with indicated no. of ovulations (%)				Uterus content parous females (%)			Uterus empty (%)	
		no.	%	%	1	2	3	⩾4	Egg	I & II instar	III instar	abortion	PL*
'Baby blue'	Tanglefoot	315	44.9	24.1	37.5	20.6	7.0	10.8	29.3	62.8	2.1	3.8	0.8
'Baby blue'	Polyisobut.LMW	280	50.6	11.4	28.9	17.1	16.4	26.1	49.2	46.4	0.4	3.6	0.0
'Baby blue'	Polyisob.HMW	637	46.2	21.5	20.3	16.6	17.1	24.5	46.0	47.0	1.2	2.0	3.2
White	Temoocid	116	41.3	15.5	22.4	19.8	20.7	21.6	46.9	51.0	1.0	0.9	0.0

* Post larviposition

green/yellow made it significantly better for trapping *G. morsitans morsitans* ,*G. pallidipes* (Green & Flint, 1986) and species of the *palpalis* group (Challier *et al.,* 1977; Gouteux *et al.,* 1981). More recently, Nevill *et al.,* (1993) reported that 'phtalogen blue' targets were likewise more attractive to *G. austeni* than white, 'baby blue' and black targets in Zululand (South Africa). The effect of 'royal blue' on the catch rate of *G. austeni* has not yet been confirmed on Zanzibar. The combined use of 'sky blue' or 'baby blue' with white on the same MP had likewise no effect on the catch rate for *G. austeni.* These observations are in contrast with those of Green (1989) who observed significant increases in catch rate of *G. p palpalis* with half 'phtalogen blue' and half white screens as compared to single blue and single white screens. However, incorporating high ultraviolet reflective white in single and two coloured targets lowered their attractiveness for *G. pallidipes* considerably (Green, 1993). Mérot & Filledier (1985) obtained increased catch rates for *G. morsitans submorsitans* with two coloured screens, half black and half blue. In general, blue is the colour mainly responsible for attracting the flies to a position close to the trap or screen. The landing response is induced by white for *G. p. palpalis* (Green, 1989) or black for *G. pallidipes* and *G. m. morsitans* (Vale, 1982; Green, 1993). The only occasion in our experiments where colour or a combination of colours increased the catch rate for *G. austeni* significantly was with LP coloured white on one panel side and blue on the other side. Significantly more flies were found sticking on the blue side of the panel which seems to indicate the superiority of blue over white for inducing landing responses of *G. austeni.* Further experiments and long term observations are required to elucidate the biological background of the colour preference and to confirm the relative importance of blue and white on the behaviour of *G. austeni* .

Ever since Swynnerton (1936) observed 91% of *G. austeni* flies landing on the legs of bait oxen, just above the hoof, *G. austeni* are considered to be low - landers. This was confirmed by the vertical distribution of the flies on the Chuka trap (Madubunyi, 1990) and our observations in this study. It therefore seems that dark shapes contrasting with a lighter background could be important for the landing behaviour of *G. austeni.* Despite the fact that single black targets were highly unattractive for *G. austeni* (Hall, 1986), the importance of black as a colour that induces a strong landing response should not be ruled out and deserves further investigation.

The good catch rate of single white MP for *G. austeni* , also observed by Hall (1986) and Madubunyi (1990), remains likewise enigmatic. White is in general an unattractive colour for other tsetse species

(Colvin & Gibson, 1992) and *G. austeni* might be exceptional in this respect. Only Lambrecht (1973) caught significantly higher numbers of *Glossina morsitans centralis* on white solid panels as compared with other colours, most likely because of its contrast with the environment.

It is well known that different sampling methods give different samples with respect to numbers, sex ratio, age composition and reproductive status of the female flies (Challier, 1982). No data are currently available on the exact sex ratio of newly emerged *G. austeni* in the field. Data from the laboratory showed that 50.6% of the emerging flies (n = 350,000) were females (Vreysen, 1992b). If the same is assumed in the field, one can however expect more female flies than male flies in the adult population owing to a sex-differential mortality of the pupae (Challier, 1982) and the longer life span of female flies (Buxton, 1955). As a result, traps in general catch more females than males (Challier, 1982). If the above is true for *G. austeni*, females were significantly under sampled with the LP in most colour combinations and during long term sampling with the MP. These data are in accordance with the observations of Madubunyi (1990) who observed female ratios of 0.14 - 0.18 with the Chuka sticky trap. However, the under sampling of females in the LP catches might have been influenced by the onset of the rains when the experiment was conducted. During periods of heavy rain, female apparent densities in the Jozani forest of Unguja drop drastically as opposed to male apparent densities. This has been associated with reduced availability of suitable larviposition sites and/or the decreased activity pattern of female flies (Vreysen, 1992a).

Non significant differences in sample size of *G. austeni* were obtained with the 3 types of sticky panels although size (Hargrove, 1980; Challier *et al.*, 1977; Dagnogo & Gouteux, 1985) and shape (Vale, 1974) are parameters which significantly influence the catch rate of trapping devices for various tsetse species. Different proportions of the *G. austeni* female fly population responded however differently to the different panel types with significantly more younger flies trapped in 3D than in MP and LP. These data suggest that shape and size might be more important for younger female flies than older flies. Significant associations between sampling device and age compositions of female flies have been recorded for several other tsetse species e.g. *G. pallidipes* (Jaenson, 1981), *G. longipennis* (Kyorku *et al.*, 1990) and *G. morsitans submorsitans* (Mohamed-Ahmed *et al.*, 1993). Movement is a factor known to influence significantly the age distribution of the sampled females with moving targets usually containing more young

females than stationary trap samples (Challier, 1982). Rotability of the 3D panel, by virtue of its 3 dimensional structure, might be better than the other panel types and could explain the observed differences in age distribution of the sampled *G. austeni* females. The differences in age distribution observed during the long term sampling with the MP, were most likely influenced by seasonal factors as the sampling was not carried out concurrently.

The availability of females with different reproductive phases for trapping is directly related to the duration of each of the physiological stages. The duration of *in utero* development of *G. morsitans* maintained at 25°C, calculated as percentage of the total duration of the reproduction cycle was 1, 42, 11, 22, and 24% for the empty uterus, egg, I, II and III instar larva respectively (Denlinger & Ma, 1974). Based upon these data, female *G. austeni* displaying an egg *in utero* were well represented (46 - 49 %) when sampled with the BBMP and WMP (except for BBMP/Tanglefoot). Parous females with immature larval stages (L1, L2) *in utero* were very well presented (46 -51%) whereas females bearing a third instar larva were under represented (0.4 - 1.2%). These data are in accordance with trap data of *G morsitans submorsitans* (Mohamed - Ahmed *et al.*, 1993) and of *G. pallidipes* (Turner, 1987) sampled with the biconical trap. Activity of *G. pallidipes* females peaks after larviposition and at the end of the second instar larva which makes this proportion of the population more available for trapping (Turner, 1987). Females with a L3 *in utero* do not feed (Challier, 1982) and are therefore less active and less available for trapping. The proportion of *G. austeni* females with a L3 *in utero* in our samples was however much lower than in catches of *palpalis* (8 - 21%) (Challier & Laveissière, 1973; Van der Vloedt *et al.*, 1980) and *morsitans* species (15%) (Mohamed - Ahmed, 1993) trapped with the biconical trap.

Of the various non-setting adhesives tested in the laboratory for their efficiency against *G. morsitans* and *G. austeni*, Ryan & Molyneux (1981) found Oecotac the most suitable product. Our field tests have conclusively demonstrated the significant effect of the used sticky material on sample size and sex ratio with Polyisobutylene.LMW being significantly better than Hyvis 2000, Oecotac A10 and Polyisobutylene.HMW. Catches with Temoocid were significantly better than with Polyisobutylene.HMW, but a comparison with Polyisobutylene.LMW could not be carried out. Especially female behaviour towards different types of sticky material was remarkable with very poor performance of the Hyvis 2000 and Oecotac A10. Although differences in sample size might partially be attributed to differences in stickiness of the various materials tested, this cannot

explain the observed differences in female ratio. Intersexual differences in olfactory responses have been detected for *G. pallidipes* for acetone, octenol, cow urine (Dransfield *et al.,* 1986), buffalo urine (Owaga, 1984) and phenolic components (Torr *et al.,* 1989). The distortion in sex ratio in our experiments could indicate that male and female *G. austeni* respond differently to some unidentified odour components of the sticky material. At present, no specific olfactory attractants have been identified for *G. austeni*, aside from a positive response to ox breath and acetone in the laboratory (Bursell, 1984) and in Mozambique (Takken, 1984). Acetone and phenols (Madubunyi, 1990) and acetone, octenol and ketones (Turner, 1984) did not increase the catch rates on Unguja for *G. austeni* with the Epsilon and biconical trap respectively. However, the negative results of these experiments were probably more related to the refusal of *G. austeni* to enter these devices than to the potential attractive effect of the odours. The forested tsetse habitats of Zanzibar are characterised by high stature trees with dense undergrowth resulting in extreme low visibility. It would therefore seem logical to assume that odours must play a significant role in host location behaviour at long or medium range. Colour of the trapping device most likely plays a significant role at close range and in the landing behaviour as evidenced by the catches of the blue and white LP.

In conclusion, the data presented in this paper demonstrate the usefulness of the sticky panel for monitoring populations of *G. austeni*. In their current shape and form, the MP and LP have several advantages over the 3D, being considerably cheaper, easier to handle and with fly samples containing more parous females. It would be worthwhile to extend this study and assess the effect of different colours and/or colour combinations more specifically in relation to attractiveness, landing and alighting behaviour. Finally, the sticky panel seems at present the most appropriate tool for testing various olfactory components in the field.

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Chapter 14

QUALITY CONTROL ASSESSMENT OF STERILE *Glossina austeni* MALES IN THE LABORATORY, DURING TRANSPORT AND AFTER RELEASE ON UNGUJA (ZANZIBAR) ISLAND

Abstract

The radiation treatments given to male *Glossina austeni* before their deployment in the field had no detectable effect on insemination potential or competitiveness. Good survival results were obtained for both treated and untreated males in transport simulation tests. During pilot release trials, a total of 22,563 sterile *G. austeni* males were transported from TTRI, Tanga to the Jozani forest, Unguja island under two different release schedules. On average, 91.6% of the males transported were actually released. Direct transport in the morning (average transport time was 2.5 ± 0.5 hours) proved to be superior (release rate of 94.7%) compared to a schedule in which flies were transported from Tanga to Zanzibar in the evening with the release being done the next morning (release rate of 85.8%). These data were corroborated during operational releases from June '92 to August '94 where more than 770,000 males were transported and released. In addition, sterile male performance was assessed in different release cages and under different marking methods. In preparation of the aerial release programme sterile males were transported in carton release boxes (25 x 10 x 5 cm) up to 200 males per box. Survival rate was comparable with the one obtained in other release containers.

Survival of sterile males was assessed in the field during release - recapture studies. Daily mortality rate fluctuated between 10 and 14% with a mean longevity of 5-7 days. The maximum period observed between release and recapture was 42 days. In virgin males, the maximum width of the apical body of the accessory glands increased from 0.183 ± 0.042 mm in 1 day old males to 0.226 ± 0.021 mm in 25 day old males. In older males, the width of the apical body decreased again. First data on accessory gland development of recaptured sterile males were accumulated. The potential of using these data to assess the competitiveness and mating frequency of released sterile males is discussed.

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Table 1. Longevity of untreated and treated *G.austeni* males (n=90), maintained in the laboratory and fed daily *in vitro* on frozen and thawed cow blood

Treatment	Average life span (Mean ± SD.) (days)(*)	Days following emergence to reach indicated % mortality		
		50	90	100
Control	77 ± 30.0 a	91	98	99
Marking	69 ± 36.4 ab	89	97	99
120 Gy Ir.	52 ± 20.2 b	54	71	96
Marking/120 Gy	58 ± 24.2 b	60	90	98

(*) data in same column followed by a common letter are not significantly different (t test, $p > 0.05$)

Table 2. Survival data for male *G.austeni* in standard release cages during transport simulation studies

No. males in release cage	Treatment	Transport	% Survival on indicated days following emergence			
			10	20	30	40
300	Control	none	96.6	93.3	81.3	68.7
300	Marking/120 Gy	none	93.4	88.2	80.6	65.8
500	Control	none	96.0	92.6	86.4	78.3
500	Marking/120 Gy	none	89.6	85.7	80.6	66.2
500	Control	3 hours	98.1	91.5	85.3	71.7
500	Marking/120 Gy*	3 hours	93.0	88.7	83.2	70.2
500	Marking/120 Gy**	3 hours	97.4	93.6	78.7	59.6
800	Control	none	97.0	94.4	90.0	86.7
800	Marking/120 Gy	none	90.0	88.1	77.4	66.3

* Marking and irradiation treatment on the same day

** Marking and irradiation treatment on 2 consecutive days

INTRODUCTION

A prerequisite for the successful implementation of a Sterile Insect Technique (SIT) programme is the production and release of high quality sterile flies. Male tsetse flies, sexually sterilised by ionising radiation need to have a life span as long as possible and be competitive with the native fly population. In addition, they should exhibit the same behaviour as native flies in order to be able to trace the wild females and successfully complete the mating act. Therefore, quality control assessment of tsetse flies, produced in the mass rearing facility and being prepared for shipment and release in the field, is an extremely important component of the entire programme. A programme, where SIT is integrated with other conventional techniques, is currently conducted to eradicate the tsetse fly *G. austeni* from the island of Unguja. The tsetse flies are mass reared at the Tsetse and Trypanosomiasis Research Institute (TTRI) at Tanga by means of the artificial membrane feeding system (Tarimo *et al.*, 1988) whereas the Tsetse Control Unit of the Department of Livestock Development on Zanzibar (DLDZ) is executing the field programme. During the first phase of the project, the biological quality of the flies was tested in the laboratory following various treatments (marking, gamma radiation, packing in release cages, transport etc..). Subsequently, the impact of transport from the rearing facility to the natural habitat was evaluated on important biological parameters. Data on fly survival after release were collected and are also presented in this paper. The size of the apical body of the accessory glands of male flies was studied in the laboratory and the field to provide indirect evidence of their mating frequency and competitiveness.

MATERIAL AND METHODS

1. QUALITY CONTROL OF STERILE MALE FLIES IN THE LABORATORY

1.1. Fertility and mean longevity of gamma treated males

Batches of male *G. austeni* (n = 90) immobilised by chilling at 4 °C shortly after emergence and marked with acrylic paint were exposed to 120 Gy radiation treatment (^{137}Cs-source at Tanga). On day 9 following emergence, 20 males of each experimental group were allowed to mate with virgin females. Female fecundity and longevity of all treated and control males was recorded.

Table 3. Competitiveness of marked/irradiated (kept at different densities in release container) and untreated *G. austeni* males [1]

No. of virgin females introduced	Male Density: 300		Male Density: 500		Male Density: 800	
	Mating pairs and type of male *in copula*		Mating pairs and type of male *in copula*		Mating pairs and type of male *in copula*	
	Untreated	Marked/ treated	Untreated	Marked/ treated	Untreated	Marked/ treated
5	3	2	3	2	2	3
5	5	0	1	4	2	3
5	4	1	2	3	3	2
5	1	4	4	1	3	2
Total	13	7	10	10	10	10

[1] 60 males (30 untreated and 30 treated) in container with 20 virgin females being added.

Table 4. Competitiveness of marked/irradiated *G.austeni* males 500 / release cage and transported for 3 hours [1]

No. of virgin females introduced	*Treatment on same day*		*Treatment on consec. days*	
	Mating pairs and type of male *in copula*		Mating pairs and type of male *in copula*	
	Untreated	Marked/ treated	Untreated	Marked/ treated
5	3	2	4	1
5	4	1	0	5
5	2	3	2	3
5	2	3	2	3
Total	11	9	8	12

[1] 60 males (30 untreated and 30 treated) in container with 20 virgin females being added.

1.2. Survival of males kept at different cage densities

Testing of cage densities was done using specially designed release containers (aluminium frame: 44 x 44 x 5 cm with netting at the bottom to allow feeding of the flies through the silicone membrane or on the flanks of a goat). Males treated as described above (i.e. marking and 120 Gy treatment) were introduced at densities of 300, 500 and 800 respectively. These flies were fed daily (except Sundays) *in vitro* (Wetzel & Luger, 1978) and their survival was monitored for 40 days. However, 30 males of each group were removed (when 9 days old) from the release cage and used for competitiveness tests with untreated males of the same age and untreated virgin females.

1.3. Transport simulation tests

During transport simulation experiments, observations focused on "500 treated males (120 Gy) per release cage". Cages were wrapped in cotton fabric drenched in water and placed in a wooden box for two trips of 1.5 hours each in a Land Rover. Immediately after the first trip, the flies were given a blood meal (on goat), transported again and then kept for further observation (40 days) under standard feeding conditions.

1.4. Accessory gland development in virgin male flies

Batches of 20 virgin male flies, aged between 1 and 32 days were dissected. The entire male reproductive system was removed from the male fly taking care not to damage the accessory glands. The glands were immediately transferred to distilled water and the maximum width of the apical body was measured under a phase-contrast microscope. Considerable variation in the dimensions of the apical body between individual flies of the same age were observed. Therefore, a correlation analysis was carried out between the dimension of the apical body and the relative size of the fly, measured as the length of the cutting edge of the hatched cell of the wing (FAO Tsetse training manual, Vol. 1, 1982).

2. QUALITY CONTROL OF STERILE MALE FLIES DURING TRANSPORT

During the initial period of the programme, all flies were packed in the standard aluminium release cages (44 x 44 x 5 cm) at densities of 300, 500 and 600 - 800 males per cage and transported in wooden transport

Table 5. Survival records of sterile male *G.austeni*, transported from TTRI, Tanga to Zanzibar

Release Schedule	Release cage type	No. males transported	Mortality		Alive Non-flyers		Males released	
			No.	%	No.	%	No.	%
EXPERIMENTAL RELEASES								
1	AC	7,971	422	5.3	705	8.8	6,844	85.9
2	AC	14,592	136	0.9	631	4.3	13,825	94.7
	TOTAL	22,563	558	2.5	1,336	5.9	20,669	91.6
OPERATIONAL RELEASES								
2	AC/PC	860,535	22,644	2.6	62,918	7.3	774,973	90.1

AC: Aluminium cages (44 x 44 x 5 cm)
PC: Plastic cages (35 x 26 x 5 cm)

boxes as described above. Only 10 minutes were required to transport the flies by Land Rover from the TTRI Institute to Tanga Airport. There, the transport boxes were loaded in a small aircraft and flown (in 35 - 40 minutes time) to Zanzibar Airport.

Several release schedules were tested:

1. Release Schedule 1 (RS 1): flies were brought to Zanzibar in the early evening, and were kept overnight inside the transport box at the laboratory of the DLDZ. Early the next morning (between 6.00 and 7.00 a.m.), flies were offered a blood meal on a goat before being transferred (45-60 minutes transfer time) to the forest.

2. Release Schedule 2 (RS 2): flies were fed *in vitro* at TTRI early in the morning (between 5.00 and 6.00 a.m.) on the day of release and after shipment by plane to Zanzibar (between 7.00 and 8.00 a.m.), immediately transported to the field and released.

Upon arrival at the release points, the number of dead flies and the proportion of flies too weak to take off was recorded.

In addition, the effect of various transport conditions on sterile male performance was assessed. Sterile male survival during the period packaging - release was compared between (1) flies transported during experimental releases and during operational releases, (2) flies transported in different types of release cages at different fly densities per cage (AC: Aluminium Release Cage with dimensions 44 x 44 x 5 cm, PC: plastic type with dimensions 35 X 26 x 5 cm), (3) flies transported in cages with and without resting space (provided through aluminium intersections), (4) flies transported in carton aerial release boxes (25 x 10 x 5 cm) as compared with flies transported in AC and PC. In addition, performance of male flies marked with Day Glo fluorescent powder was assessed. This marking technique was introduced as an alternative for the thorax marking with acrylic paint in order to reduce the handling of the flies. Pupae are buried under dry sieved sterile sand that has been mixed with 0.25% of fluorescent dye (Williamson *et al.*, 1983b). After emerging from the pupae, the flies inflate their ptilinum and dig their way through the sand. The ptilinum picks up small quantities of dye and when the ptilinum is retracted, the dye is sequestered inside the frontal suture. Recaptured flies can be identified by crushing their heads on a filter paper and examination in a

Table 6. Survival records of sterile male *G.austeni*, transported from TTRI, Tanga to Zanzibar under different transport conditions

Release Schedule	Release cage type	Male density in release cage	No. males transported	Mortality		Alive Non-flyers		Males released	
				No.	%	No.	%	No.	%
EXPERIMENTAL RELEASES									
1	AC	300	600	49	8.2	31	5.2	520	86.7
1	AC	500	4,485	221	4.9	416	9.3	3,848	85.8
1	AC	600-800	2,886	152	5.3	258	8.9	2,476	85.8
2	AC	300	2,067	23	1.1	114	5.5	1,930	93.4
2	AC	500	4,500	34	0.8	161	3.6	4,305	95.7
2	AC	600-800	8,025	79	2.0	365	4.6	7,581	94.5
OPERATIONAL RELEASES									
2	AC	800	49,817	791	1.5	3,270	6.6	45,756	91.8
2	AC	925-1050	45,505	591	1.2	3,199	7.0	41,715	91.7
2	PC	300	22,216	485	2.1	1,902	8.6	19,829	89.3
2	PC	350-400	3,375	86	2.5	399	11.8	2,890	85.6
2	PC	300	5,400	130	2.4	492	9.1	4,778	88.5
2	PC*	300	5,400	153	2.8	525	9.7	4,722	87.4

AC: Aluminium cages (44 x 44 x 5 cm)
PC: Plastic cages (35 x 26 x 5 cm)
* Plastic cages with aluminium intersections

dark room with UV light under a normal dissection microscope or under a more sensitive fluorescence microscope.

3. QUALITY CONTROL OF STERILE MALE FLIES AFTER RELEASE

3.1. Working area and survey techniques

All field observations were carried out in the Jozani forest. This primary forest is situated between 6°15'S - 6°16'S and 39°24'E - 39°25' E and comprises a forest reserve covering approximately 10 km^2, with an important northwest extension towards Kisomanga, and in the Northeast an extension towards Charawe (chapter 12, Fig. 1). Sampling of the flies was done by means of a sticky panel (Hall, 1986; Madubunyi, 1989; Hall, 1990; Schönefeld, 1988; Vreysen, chapter 13). All panel types (monopanel (MP) (Vreysen, 1992), 3-Dimensional Targets (3-D) (Schönefeld, 1988) and legpanel (LP)(Chapter 13)) were constructed from 4 mm thick plywood, fibreglass or PVC. MP and 3-D panels were painted with two layers of "Baby Blue" or white water resistant enamel paint. LP were painted on one side with sky blue and the other side with white paint. Temoocid or Isopolybuthylene was applied on both sides of the panel as a sticky substance. All panels were suspended with a rope and hung from an overhanging branch permitting free rotation with the slightest breeze.

3.2. Field cage survival tests

Samples of untreated control and 120 Gy treated males of different age (1-2, 4 and 8 days old), kept in standard colony holding cages (11 cm diameter, 4.5 cm high) at a density of 30 males per cage, were transported from TTRI, Tanga to the Jozani forest together with the normal release containers in the wooden transport boxes. The cages were suspended with a string under a tree branch in the middle area of the forest i.e. a site with good shade, moderate temperatures and high humidity. The supporting branch was encircled with a sticky substance to prevent invasion of ants. After suspension, the flies were not fed again. Survival in the cages was recorded daily until all flies had died. Each experiment was replicated 3 times.

Table 7. Survival records of sterile male *G.austeni*, transported in carton aerial release boxes, aluminium and plastic release cages from 15 July to 19 August '94

Marking	No. males transported	Mortality		Alive Non-flyers		Males released	
		No.	%	No.	%	No.	%
Carton boxes	28,600	677	2.4	1,073	3.8	26,850	93.8
Release cages	94,435	1,430	1.5	3,475	3.7	89,451	94.8

Table 8. Survival records of differently marked sterile male *G.austeni*, transported from TTRI, Tanga to Zanzibar

Marking	No. males transported	Mortality		Alive Non-flyers		Males released	
		No.	%	No.	%	No.	%
No marking	118,145	2,284	1.9	6,304	5.3	109,557	92.7
Thorax marking	449,347	13,471	3.0	40,133	8.9	395,743	88.1
Day glo marking	132,218	2,714	2.1	5,851	4.4	123,653	93.5

3.3. Survival of sterile males in the forest habitat

Survival of the released male flies was assessed during two periods i.e.:

- from 29 November 1990 to 3 January 1991, a total of 8,772 sterile male flies were released during 6 release sessions from two release points. Trapping was carried out daily in 74 trapping sites until no more sterile flies were caught. Data for the six releases were pooled.

- on 1, 8 and 15 October 1993, a total of 21,980 sterile males were released during 3 release sessions in 9 release points. Trapping was carried out in 8 monitoring sites every week for 2 consecutive days for a total of 9 weeks.

RESULTS

1. PERFORMANCE OF STERILE FLIES IN THE LABORATORY

The average life span and trends in longevity for control males, for colour marked males, for 120 Gy treated males and for marked and radiation treated males is given in Table 1. Thorax marking with acrylic paint and irradiation reduced the average life span of the males significantly with 9.9 and 24.5 days respectively (t test, $p < 0.05$), but a combined treatment had no further negative impact on their survival. The mean longevity of all treated males remained above 50 days. These survival results were comparable with data obtained for other species (Chapter 2). All matings resulted in normal insemination and normal fecundity except for females mated to the radiation sterilised males, which did not produce any pupae.

Survival data of differently treated males and caged at various densities are summarised in Table 2. Insignificant differences in mortality trends were found during 40 days of observation. Moreover, the data on the performance of males taken out of the release containers and used in competitiveness tests (Tables 3 and 4) showed normal vigour of the treated males, independent of cage density and transport.

Table 9. Survival of unfed untreated and treated *Glossina austeni* males, kept in standard holding cages suspended in the Jozani forest

Age of males during transport (days)	Treatment	Males No.	Average longevity (days ± SD)	Maximum recorded survival (days)	% survival on indicated days following transport					
					2	4	6	8	10	13
1-2	Control	120	5.8 ± 2.4	13	100	81.6	38.3	17.5	12.5	0.8
	120 Gy	120	6.5 ± 2.5	12	97.5	88.3	48.3	30.8	20.8	0.0
4	Control	120	6.2 ± 2.5	15	97.7	93.3	42.2	21.1	14.4	2.2
	120 Gy	120	6.3 ± 2.3	12	98.8	91.0	50.5	23.5	14.6	0.0
8	Control	120	6.4 ± 2.9	18	94.7	91.5	53.3	27.1	21.1	4.2
	120 Gy	120	5.8 ± 2.7	15	94.0	86.4	45.7	23.7	15.2	1.7

2. PERFORMANCE OF STERILE FLIES DURING TRANSPORT

During 10 experimental releases, 22,563 sterile males were transported from Tanga to Jozani, Unguja of which 20,669 (91.6%) were actually released (Table 5). The fitness of the males transported was more affected (5.3% mortality plus 8.8% alive non-flyers) under RS 1 than under RS 2 (0.9% mortality plus 4.3% alive non-flyers). During operational releases, 860,535 sterile males were transported under RS 2 from June '92 to August '94 of which 774,973 (90.1%) were released from the ground (Table 5). The increase in the number of flies handled per release session resulted in a higher mortality rate (2.6%) and an increased proportion of live non-flyers (7.3%).

Table 6 summarises the data of males transported in different densities during the experimental releases. The findings corroborate the results of the cage density tests carried out under laboratory conditions i.e. no differences in survival rate of the sterile males were found for both release schedules, irrespective of the cage density (from 300 to 800). Data accumulated during operational releases indicate superior performance of the male flies transported in aluminium release cages (AC)(44 x 44 x 5 cm) as compared to plastic release cages (PC) (35 x 26 x 5 cm) (release rate of 91.8% versus 89.3 and 85.6%, $p < 0.05$). Increasing the fly density in the AC from 800 to 1000 (decreasing the available space of 1 fly from 12.1 cm^3 to 9.6 cm^3) had no impact on fly quality. An increase from 300 to 400 flies in the PC however (decrease of available space from 15 cm^3 to 11.3 cm^3 per fly) reduced the performance significantly from 89.3 to 85.6%. (chi square, $p < 0.01$). Providing additional resting space for the flies by installing aluminium intersections in the PC had no influence on fly survival (chi square, $p > 0.05$).

Male fly survival and proportion of weak flies was comparable for sterile males released from carton aerial release boxes (200 males per box) and in aluminium and plastic release containers (Table 7). The effect of different marking techniques on the performance of the flies is presented in Table 8. Quality of sterile males marked with Day Glo and unmarked flies was similar. Flies marked with acrylic paint on the thorax survived less well (88.1%).

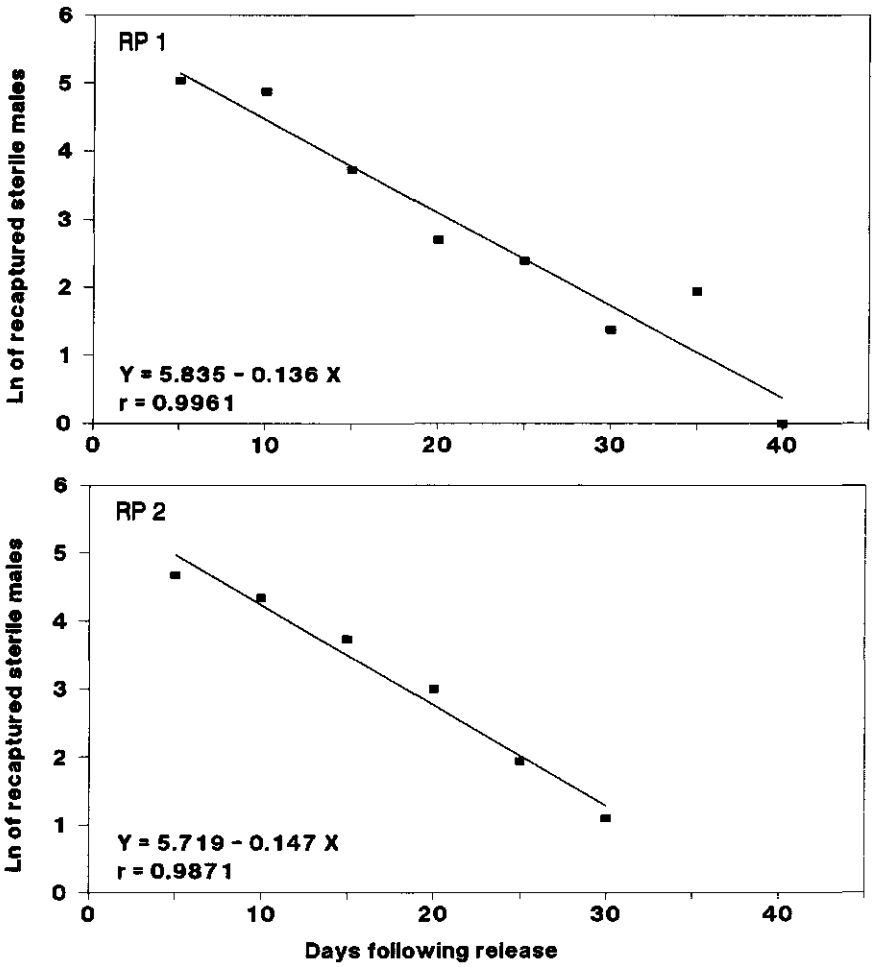

Fig. 1 Survival of sterile males released during the period November '90 - January '91 in RP 1 and RP 2 of the Jozani forest (data combined for 6 releases).

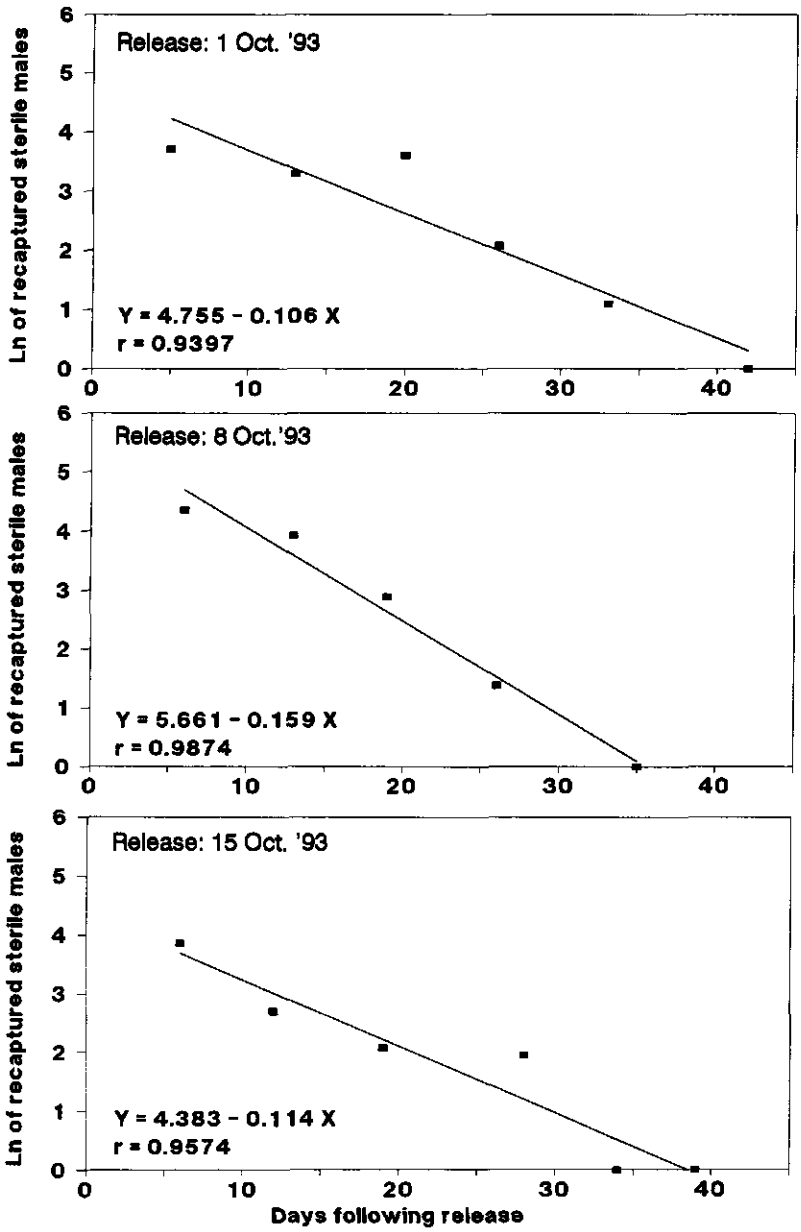

Fig. 2 Survival of sterile males released in 3 release sessions from 1 to 15 October '93.

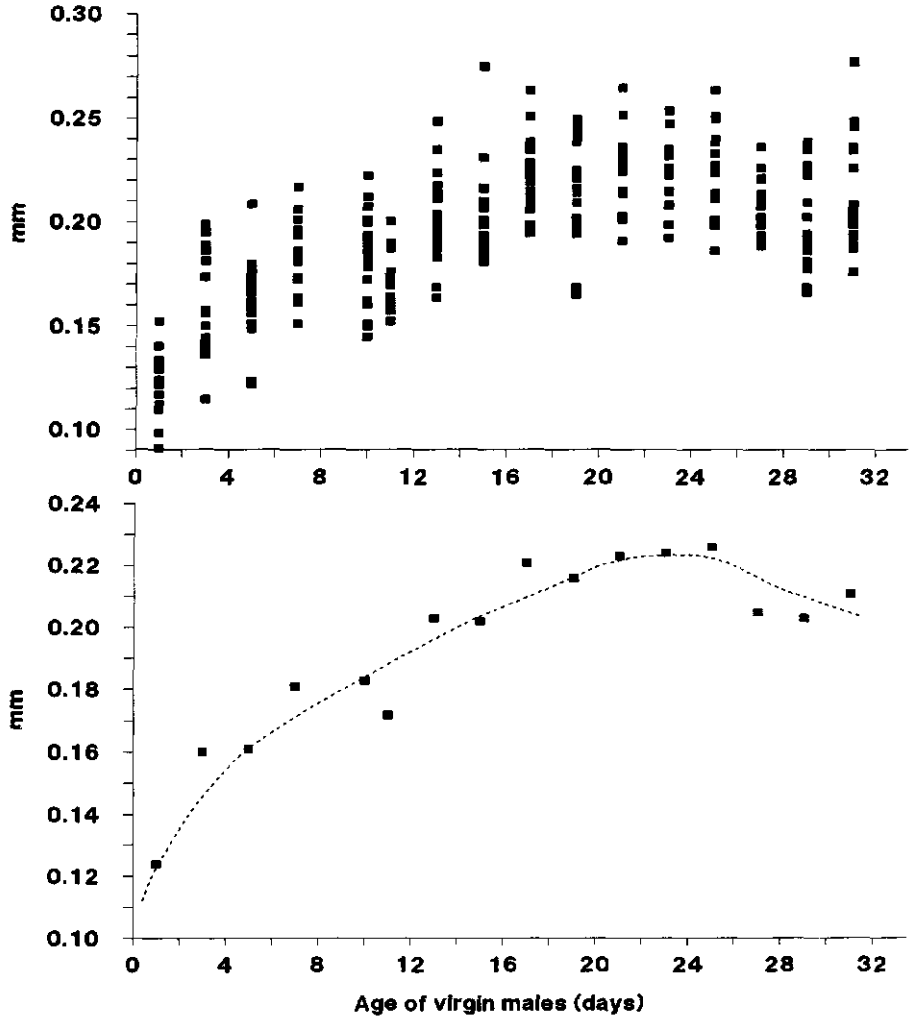

Fig. 3 The variation of the width of the apical body of the accessory glands of virgin *G. austeni* males, aged between 1 and 31 days (top graph) and the average width of the apical body of the accessory glands plotted against the age of virgin *G. austeni* males (bottom graph).

3. PERFORMANCE OF STERILE FLIES AFTER RELEASE

3.1. Field cage survival tests

Pooled data of the survival rates are presented in Table 9. Untreated control males, aged 1-2, 4 and 8 days, lived on average 5.8 ± 2.4 days, 6.2 ± 2.5 days and 6.4 ± 2.9 days respectively ($p > 0.05$). No significant differences in average longevity were observed between the different experimental groups (mean life spans of 6.5 ± 2.5 days, 6.3 ± 2.3 days day 4 after transport and 20 % were still alive on day 8. Maximum recorded survival however increased with increasing male age during transport, with a recorded 13, 15 and 18 days for control males aged 1 - 2, 4 and 8 days and 12, 12 and 15 days for the treated males respectively.

3.2. Survival of released sterile males

During the period November 1990 - January 1991 (P1) and October '93 (P2) a total of 8,772 and 21,980 sterile males were released in 6 and 3 release sessions respectively. A total of 624 sterile males (7.1%), released during P1, were recaptured with the maximum period between release and recapture fluctuating between 18 and 37 days. During the various trapping sessions following the release of P2, a total of 117 (1.3%), 152 (2.4%) and 80 (1.1%) sterile males were recaptured with a maximum period between release and recapture of 42 days, 35 days and 39 days for the 3 releases, respectively.

A measure of the average survival and daily mortality rate can be obtained from the declining rate of recapture with time after a particular release (Bourne, 1982). Fig. 1 presents the trapping results (the numbers of recaptures plotted to the base "e" on successive trapping occasions) after the 6 releases during P1 in 2 release points, whereas Fig. 2 presents the recapture data of the three releases during P2. The slope of the regression line indicates the finite rate of decline i.e. daily mortality. The estimated daily loss of the flies released in P1 was 12.7% and 13.7% for males released at the two release points respectively with a mean longevity (time after which 50% of the released flies have died) of 5.1 and 4.7 days. Estimated daily losses of the sterile males released during P2 were 10.0%, 14.6% and 10.7% for the three releases respectively. The average longevity was calculated as 6.5 days, 4.4 days and 6.1 days for the males released in the 3 sessions respectively. Based upon these data, an estimation could be

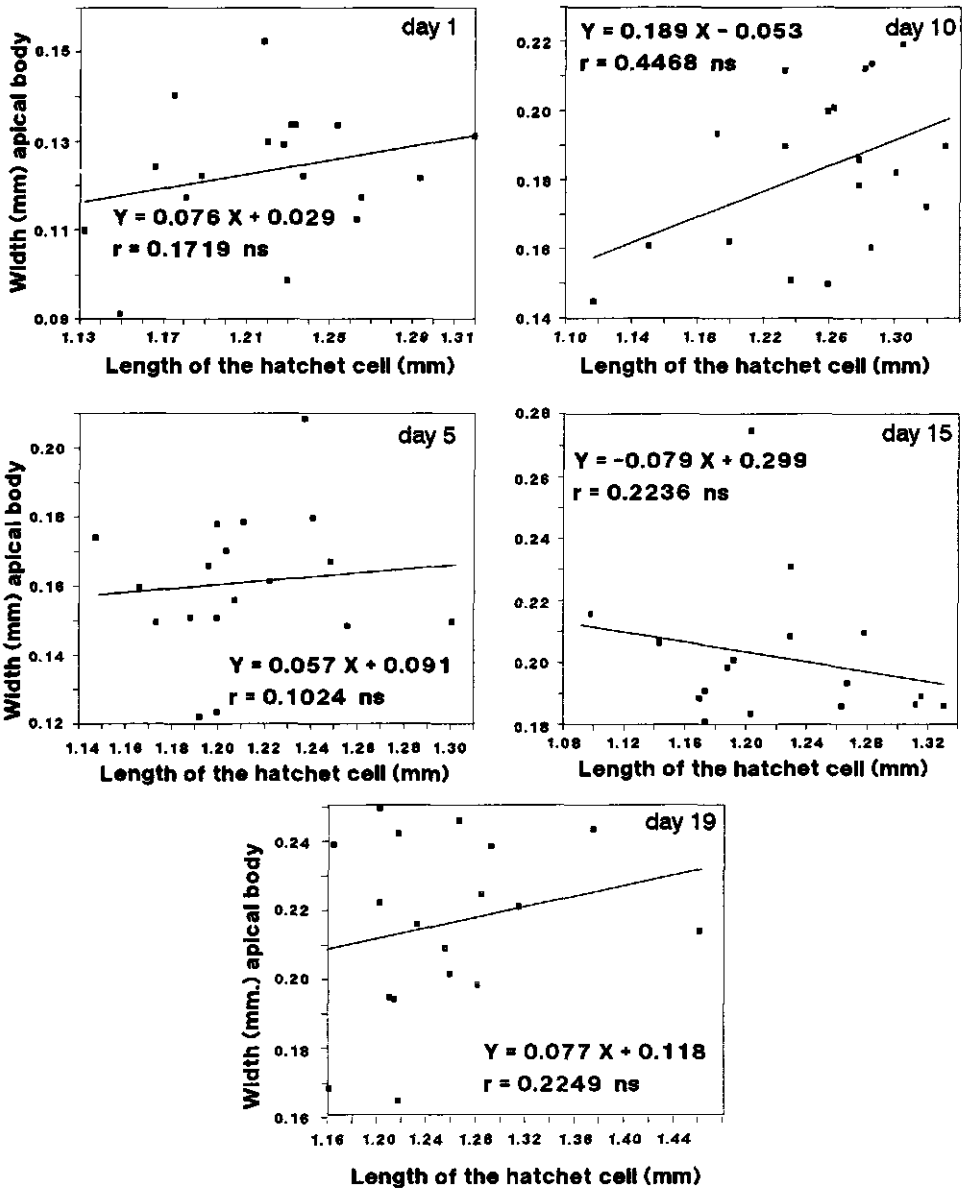

Fig. 4 The width of the apical body of the accessory glands of virgin *G. austeni* males, aged 1 - 5 - 10 - 15 and 19 days, plotted against the length of the cutting edge of the hatched cell of the wing.

made on the amount of sterile males alive in the forest on any given day. Assuming a 10% daily mortality rate, an average of 6,200 and 12,400 sterile males will be alive in the forest on the fourth day after release with weekly releases of 5,000 and 10,000 sterile males respectively.

3.3. Development of the apical body of the accessory glands in sterile males

3.3.1. The apical body width in virgin males of increasing age

Fig. 3 presents the variation of the apical body width with the age of virgin *G. austeni* males. The width of the apical body of 1 day old virgin males was on average 0.124 ± 0.015 mm (range 0.09 - 0.15 mm) and increased to 0.183 ± 0.042 mm and 0.221 ± 0.018 mm in 10 and 19 day old males respectively. The maximum apical body width was reached in males aged between 21 and 25 days old (average width of the apical body of 0.223 ± 0.020 mm, 0.224 ± 0.019 mm and 0.226 ± 0.021 mm for 21 day old, 23 day old and 25 day old virgin males respectively). A decline in average apical body width was observed in males older than 25 days (average between 0.203 mm. and 0.211 mm).

Fig. 4 presents the scatter diagrams of the width of the apical body of the accessory glands of virgin males aged between 1 and 19 days in relation to the relative size of the male flies measured as the length of the cutting edge of the hatchet cell of the wing. The data show that the width of the apical body of the accessory glands was not significantly correlated with the size of the fly in all age groups except for 7 day old flies ($r = 0.5887$, $p < 0.05$).

3.3.2. The apical body width in released - recaptured males

The age of male *G. austeni* released in the Jozani forest ranged between 2 and 7 days. Monitoring activities were never initiated before the 4 Th. day after release. Therefore, the youngest sterile males recaptured were at least 6 days old. The average width of the apical body of 5 - 7 day old virgin males was 0.16 - 0.18 mm (Fig. 3). Taking into account the variations in apical body width in virgin males of the same age, it can be assumed that recaptured males with an apical body dimension of < 0.14 mm display depleted glands or glands in the process of replenishment and should have mated at least once. Table 10 summarises the average width of the apical body of 279 and 116 sterile males, recaptured between day 4 and 40 after being released during the

Table 10. The width of the apical body of accessory glands of recaptured sterile males in the Jozani forest

Month	No. sterile males	Days between release and recapture	Average width of apical body (± SD.) (mm)	Males with depleted glands (< 0.14 mm) %
September - October 1993	118	4 - 6	0.156 ± 0.030	26.4
	65	11 - 13	0.163 ± 0.034	19.9
	66	18 - 20	0.164 ± 0.037	33.3
	30	25 - 27	0.172 ± 0.037	19.9
November - December 1993	58	4 - 6	0.169 ± 0.028	12.0
	45	11 - 13	0.190 ± 0.030	6.6
	44	18 - 20	0.176 ± 0.034	13.6
	19	26 - 40	0.182 ± 0.035	15.9

period September - October '93 and November - December '93 respectively. During the first period, the average width of the apical body increased from 0.156 ± 0.030 mm for males recaptured 4-6 days after release, to 0.163 ± 0.034 mm ($p > 0.05$), 0.164 ± 0.037 mm ($p > 0.05$) and 0.172 ± 0.037 mm ($p < 0.05$) for males recaptured 11 -13 days, 18 - 20 days and 25 - 27 days after release respectively. The average width of the apical body of male flies released during the second period increased from 0.169 ± 0.028 mm for sterile males recaptured between 4 and 6 days following release to 0.190 ± 0.030 mm ($p < 0.05$) for males recaptured between 11 and 13 days after release. The width of the apical body decreased again in older flies to 0.176 ± 0.034 mm (recaptured 18 - 20 days after release) ($p > 0.05$) and 0.182 ± 0.035 mm ($p < 0.05$)(recaptured 26 - 40 days after release). The average width of the apical body of the youngest males (4 to 13 days between release and recapture) was significantly different for the two observation periods (t-test, $p < 0.01$) with 46.3% and 18.6% of the recaptured males displaying apical bodies with dimensions < 0.14 mm.

DISCUSSION

The potential use of the Sterile Insect Technique for the elimination of a tsetse target species was first demonstrated in Tanzania against *Glossina morsitans morsitans* in the seventies. (Williamson *et al.* 1983 a,b,c,d). The introduction of the *in vitro* feeding technology a decade later (Bauer, *et al.,* 1984; Wetzel & Luger, 1978; Van der Vloedt *et al.* ,1987) disposed of the cumbersome feeding of tsetse flies on live animals and culminated in the maintenance of tsetse fly colonies of unprecedented size (80,000 to 100,000 producing females). This paved the way to the successful eradication of *G. p. gambiensis, G. tachinoides* and *G. morsitans submorsitans* from 3,500 km^2 in Burkina Faso (Politzar & Cuisance, 1984) and *G. p. palpalis* from 1,500 km^2 in Nigeria (Takken *et al.,* 1986; Oladunmade *et al.,* 1990). The success of the SIT component in both programmes can largely be attributed to the persistent production of a sufficient amount of high quality male flies. This was achieved by adequate sterilisation, marking and packing techniques and the development of transport and release methods which did not negatively affect the sterile males' fitness once released in the field. Moreover, no assortative matings between the colonised strain and the wild strain was observed which led for instance, to the failure of several mosquito SIT programmes (Weidhaas & Patterson, 1982).

Any insect control programme with a SIT component aiming at the total elimination of the target species will fail if the quality of the sterile flies is not satisfactory. In the initial phase of the Zanzibar project, experiments were initiated in the laboratory to assess the performance of male flies following various treatments and handling procedures. It was demonstrated that the marking with acrylic paint on the thorax, sterilisation of the adult male flies with 120 Gy in a gamma source, packing of flies in release cages of different materials and size at different densities produced sterile flies of acceptable quality, even during transport simulation tests. Based upon these initial findings, a transport method was developed and its suitability assessed first during experimental releases and after that during two years of operational releases. A total of 860,535 sterile males were transported with more than 90% of the transported flies actually released. Thorax marking however, affected the male quality dramatically when the amount of flies to be handled exceeded 7,000 per week. Therefore, the Day Glo Fluorescent Powder marking technique was adopted (Williamson *et al.*, 1983) without affecting female productivity in the colony and male fly performance. The same observations were recently made by Ajagbonna (1993) *with G. p. palpalis* and Djiteye (1993) with *G. austeni.* During the releases, a persistent proportion (7%) of the transported sterile male flies were alive upon arrival at the release site but refused to take off. The reason for this is not clear i.e. are these flies physically to weak to fly off or are they just inhibited to do so at the moment of release. Research should be carried out to investigate the nature of this phenomenon and disclose if there is a behavioural or a physiological significance. It would therefore be worthwhile to insert a phase in the mass rearing procedures where fly performance is assessed. In order to select against non-flyers flies should be allowed to fly from one area to an other after emergence; a procedure routinely applied in Codling Moth mass rearing (Dyck & Gardiner, 1992; Dyck *et al.,* 1993).

Performance of the sterile male flies in the field was first assessed by survival data in field cage tests. These results showed that the majority (> 80%) of the flies survived on their fat reserves for the first 2- 3 days, indicating adequate feeding before deployment in the field. This does not indicate the actual survival of the released flies but gives an indication of the potential period of time available for the released flies to find a host. Analysis of the survival of released flies in their habitat based upon release - recapture data showed a daily mortality rate of 10 - 14%. Performance of the flies in the initial phase of the programme (November '90) was inferior as compared to the one

after 2 years of releases (October '93). This is most likely a reflection of improved handling methods by a more experienced staff. Although it is difficult to compare performance of different species in different habitats, it is interesting to note that survival of the *G. austeni* males was considerably better than survival of 120 Gy treated *G. p. palpalis* males released in the riverine forests in the Lafia area in Nigeria (daily mortality of 15 - 20%, with a maximum recorded survival of 27 days (Bourne, 1982)). The estimated mean life span of *G. morsitans morsitans* released in Mwkaja Ranch in Tanzania was 8.3 days with a maximum age of 68 days (Williamson *et al.* 1983 d). Average survival of 110 Gy and 150 Gy treated *G. palpalis gambiensis* males was 9 (Politzar *et al.*, 1979) and 8 days (Clair *et al.*, 1976) with a maximum recorded age of 30.6 days. Data on survival of wild *G. austeni* are unfortunately not available. Capture-release-recapture studies were not feasible due to the applied trapping method with sticky non-setting adhesives. Behaviour, survival and dispersal of the sterile male flies could therefore not be compared with those of wild insects. It is however interesting to note that Hargrove (1981) calculated a maximum average daily female mortality of 3.5%. Mortality of released *G. morsitans morsitans* males was higher and dependant on the age of the males i.e. daily mortality of 8.3%, 5.5% and 10% immediately after emergence, at the age of 9 and 30 days respectively (Hargrove, 1990). The higher daily mortality rate of the released *G. austeni* was most likely related to the life span reducing 120 Gy irradiation treatment.

In general, the progress of a sterile male release programme can be monitored using biological evaluation methods based upon the rate of induced sterility in the sampled female flies (Van der Vloedt & Barnor, 1984). This technique, however, requires a female sample size large enough to make a sound evaluation feasible, a situation which becomes more critical when one approaches the end of an eradication campaign (Taze *et al.*, 1977). Field data on mating frequency of the male flies, based upon the proportion of male flies with accumulated accessory gland secretions and/or with depleted glands, of indigenous and released sterile males could provide complementary information on the degree of control achieved (Pollock, 1974). When no more wild flies are captured, this could still provide information on any mating going on in the field. Our base-line data on the development of the apical body in virgin males indicate a continuous increase in apical body width up to day 25. In older males, the apical body width decreased again. These data are different from the observations made by Pollock (1974) who observed a continuous increase in apical body width up to the age of 35 days.

The average width of the apical body of the sample of recaptured sterile flies increased with increasing age of the released males. This seems to indicate that (1) males exhibit reduced sexual activity, (2) become less competitive or (3) are unable to trace wild females with older age. In addition, the data accumulated over a 4 month period showed distinct differences especially in the young fly group (i.e. flies recaptured 4 to 13 days after release and assumed to be sexually most active). The higher proportion of males with depleted glands trapped in the period Sept.- Oct.' 93 as compared to males trapped in Nov. - Dec. '93 (18.6% versus 46.3%) seems to suggest reduced competitiveness of the released males during the last two months or reduced mating opportunity. These results were in accordance with the observations made on the reduced quality and survival of the males during transport since November '93.

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Chapter 15

GENERAL DISCUSSION

The research presented in this thesis has been discussed in detail in previous chapters. In this last chapter, the relevance of the main findings will be discussed in relation to the use in operational SIT programmes. The impact of the releases of sterile male flies will be discussed and an evaluation of field data on Unguja island is presented.

Radiation research on adult flies and pupae

The exposure to irradiation (or chemicals) for sterilising male insects is the basis for eradication of pest insects by means of the Sterile Insect Technique (Knipling, 1955). The reproductive capacity of tsetse flies after receiving a radiation dose is mainly affected by the induction of dominant lethals in the male germ cells i.e. not the destruction of male gonads (Van Borstel, 1962) and the lethal effects on the throphocytes for females (Nothel, 1968). The success of the method depends primarily on reduced fertility, high insemination capacity, sufficient vigour and life expectancy of the released insects (Ducoff, 1972). In male tsetse flies, dominant lethals can be induced either in adult male flies (Itard, 1968; Offori & Czock, 1975; Taze *et al.*, 1977; Van der Vloedt *et al.*, 1978) or in the late pupal phase (Dean & Wortham, 1969; Langley *et al.*, 1974; Van der Vloedt *et al.*, 1976; Williamson *et al.*, 1983b). In chapter 2, the effects of irradiation on adult male flies of 3 economic important tsetse species (*G. tachinoides, G. fuscipes fuscipes* and *G. brevipalpis*) were investigated. Interspecific differences were observed with respect to (1) radiosensitivity i.e. *G. brevipalpis* was the most radiosensitive with 50 Gy being sufficient to induce 95% sterility versus 80 Gy and 120 Gy for *G. f. fuscipes* and *G. tachinoides.* respectively, (2) viability of offspring and (3) somatic damage as expressed by adult life span. As is observed for most insect species (Ducoff, 1972), average life span of the three species decreased with increasing treatment doses, but for the same treatment dose, the life expectancy of *G. brevipalpis* was significantly higher as compared to the two other species. The significance for operational release programmes is dual: on the one hand, released sterile male flies should have a life span as long as possible to

increase the chances of encountering a receptive female (Curtis & Langley, 1982), on the other hand, the probability of developing mature *vivax* and *congolense* infections increases with longer life spans. Moreover, some scientists even claim that tsetse flies can become infected with *T. vivax* and *T. congolense* throughout their adult life (Molyneux *et al.* 1982). If this is true, the choice between releasing pupae or adult flies becomes irrelevant and the only important parameters are ambient temperature and average longevity. With a minimum development time of 12 days at 22 °C and 5 days at 29 °C for *T. vivax* and minimum development time of 19 days for *T. congolense,* consideration should be given to the average temperature and the time required for a mature infection to develop in the planning of the release strategy. Moreover, changes in the midgut structure of irradiated male flies (loss of epithelial microvilli, atrophy of epithelial cells of the mid gut and destruction of muscle cells) together with the increase in ricketssia-like particles might increase the susceptibility of the flies for trypanosome infections (Stiles *et al.,* 1989). To avoid an increased incidence of trypanosomiasis due to the release of sterile male flies, it has been suggested to release non-teneral flies free of ricketssia like organisms (Stiles *et al.,* 1989) which have been fed on blood containing trypanocidal drugs like Samorin (isometamidium chloride). The drugs will completely suppress the development of mature *T. vivax, T. congolense* and *T. b. brucei* in released sterile flies (Moloo & Kamunya, 1987).

In addition, attention should be given to the discrepancy between the survival rates of irradiated male flies in the laboratory (> than 30 days with a dose of 120 Gy as seen in chapter 2) and the ones obtained in the field i.e. average survival of 7 - 9 days for released *G. p. gambiensis* , *G. p. palpalis, G. m. morsitans* (Cuisance *et al.,* 1980; Bourne, 1982; Williamson *et al.,* 1983d) and for released *G. austeni* (Chapter 14). The reasons are most likely related to predation, difficulties in finding a suitable host and increased activity pattern (search for hosts, female mates, resting sites etc..) as compared to laboratory kept flies. However, it is unlikely that those flies live long enough to develop a mature infection and transmit the disease (Molyneux *et al.,* 1982).

Mating behaviour and sperm transfer of the three species was not affected and no sperm inactivation or aspermia was observed as a result from radiation, even with doses up to 200 Gy. This observation is relevant for the execution of SIT programmes as tsetse flies are not strictly monogamous (Jordan, 1958; Chapter 6 and 7) and a sterile mating without sperm transfer would be negated by future matings with a fertile male (LaChance *et al.,* 1967). These observations were in

accordance with those of other Diptera like *Aedes aegypti, Drosophila melanogaster* etc. (LaChance *et al.*, 1967).

Treating flies in the pupal stage has several advantages such as reduced handling, pupae are less susceptible to damage, require less space (Van der Vloedt *et al.,* 1976), possibility of bulk irradiation without chilling (Dame, 1970) and better quality flies (Curtis & Langley, 1972). Separation of the sexes in the pupal stage is not feasible as yet which prevents treatment of only one sex. In addition, releasing pupae does not permit pre-release feeding, which is advantageous for survival in the first two days following release and reduces the vectorial capacity for transmission of trypanosomes: the option where there is human sleeping sickness (Molyneux *et al.,* 1982). Bulk irradiation of adult flies is possible, as is shown in the current programme on Zanzibar, but requires additional chilling which might reduce the viability of the released flies. From a biological point of view, the drastic reorganisation paired with high mitotic activity, the most radiosensitive stages and which takes place during the process of metamorphosis, makes the time interval for the irradiation of pupae much more critical as compared to the treatment of adult flies (Ducoff, 1972). Moreover, the entire process of spermatogenesis occurs in male pupae during the mid pupal phase between day 6 and day 21 after larviposition (Itard, 1970). The high susceptibility of pupae for radiation was already demonstrated by Langley *et al.,* (1974), who determined 27 days to be the youngest age for the treatment of *G. m. morsitans* pupae. Our data with *G. tachinoides* pupae, presented in chapter 3, showed that irradiation of pupae of this species is likewise limited to the last phase of the pupal development, with 20 day old pupae being the youngest to support a treatment resulting in adequate sterility and adult viability. Treating pupae in younger stages resulted in early pupal death, reduced viability during the first days following emergence and inferior insemination capacity. However, as is shown in chapter 4, the treatment age of pupae could be reduced provided irradiation was carried out in a nitrogen atmosphere and the dose was split into two fractions. Efforts to further increase the time between treatment and eclosion of the flies by combining a radiation treatment with cooling of the pupae were disappointing. The viability of the flies was in most cases significantly reduced. The significance of these results for operational programmes is related to the concept of regional mass rearing centres. Economic analysis of the SIT programme in Burkina Faso has revealed that 70% of the costs are related to initial project infrastructure, 18% for field operations and 12% to run the

breeding during operational releases (Brandl, 1988). Significant cost reduction can be achieved by limiting the number of breeding centres, where several species could be reared and the pupae transported (irradiated for those countries lacking a radiation source) to the target areas. The importance of an adequate time frame between treatment of the pupae and eclosion is obvious. The manipulation techniques tested in this thesis have shown that pupae can be irradiated resulting in sterile, high quality flies with a period of 16 to 25 days between treatment and eclosion. Moreover, recent studies have indicated that chilling pupae at 15 °C (the temperature used in our work) for 5 days prior to irradiation reduced the infection rate of *T. vivax* in *G. p. gambiensis* (Djiteye *et al.*, 1993).

Hybridisation of closely related tsetse species

Hybridisation or non-assortative mating between closely related tsetse species or subspecies and the resulting male hybrid sterility can be used to introduce sterility in a target population (Itard, 1974). The major cause of the hybrid male sterility is the incompatibility of the X and Y chromosome and/or intrachromosomal recombination for *G. p. palpalis* - *G. p. gambiensis* and for *G. m. morsitans* - *G. m. submorsitans* and for *G. m. morsitans* and *G. m. centralis* (Curtis, 1972; Rawlings, 1985; Gooding, 1987; Gooding, 1988; Gooding, 1992). The release of hybrids has certainly potential in some situations like in the *Gpp*-*Gpg* model where all F_1 male hybrids are completely sterile (chapter 8). Sterility was however an expression of the lack of mature sperm and monogamy should therefore be induced in the virgin female after mating with a hybrid. In other models, the F_1 sterility is not sufficient and the use of the hybrids doubtful for control purposes i.e. 20% fertility in the *morsitans-submorsitans* model. The competitiveness of these hybrid males in a natural habitat and their success in tracing females remains engimatic and requires further investigation. A negative aspect of the concept of releasing sterile hybrid males as genetic control agents is the need of maintaining 2 colonies for the production of hybrids (Gooding, 1992).

An alternative is the release of satyrs (a male willing to mate with heterospecific or heterosubspecific females (Ribeiro, 1988)) resulting in no or reduced offspring. This would be a valid method for some models like (1) the release of male *G. m. submorsitans* in *G. m. morsitans* habitat i.e. transfer of *G. m. submorsitans* sperm does occur but there is no utilisation of the sperm by the female *G. m. morsitans* and consequently no insemination occurs or (2) release of *Gpg* males in

a *Gpp* habitat with resulting reduced fertility in the *Gpp* females (chapter 8), or (3) the release of *Gpp* males in a *Gff* habitat with a mating resulting in the death of the *Gff* females (chapter 10). The use of satyrs seems a valid option in some models, but the issue of selective sperm use after multiple mating requires further attention (Gooding, 1992). The work described in chapter 8 and 10, concentrated on the mating efficiency of satyrs of the *palpalis* group with females of the target species. Emphasis was put on experiments conducted in large laboratory cages in an attempt to simulate more natural conditions. Under these conditions, the absence of assortative mating between *G. p. palpalis* and *G. p. gambiensis* (Gooding, 1988) and *G. p. palpalis* and *G. f. fuscipes* (Vanderplank, 1948) was clearly demonstrated. In addition, it was proposed to combine the high hybridisation potential of the *palpalis* subspecies with the use of gamma irradiated males. The feasibility of this was demonstrated during several experiments, revealing a good mating response, sperm transfer and insemination resulting in embryonic arrest in cross matings with gamma irradiated males of both subspecies. In a cage experiment with equal numbers of males of both subspecies, it was shown that a gradual increase in the proportion of gamma irradiated males of one subspecies, proportionally increased the rate of induced sterility in the virgin female population of the other subspecies. The release of gamma irradiated flies of one subspecies in the habitat of the other has some obvious advantages: (1) the economic benefits arising from the need to rear only one subspecies, (2) government restrictions on the introduction of fertile satyrs in their country are not valid, (3) radiation treatment will reduce average life span of released satyrs, reducing the risk of developing mature trypanosome infections if released.

The impact of sterile male releases on Unguja island

The eradication campaign on the island of Unguja is the first to be conducted against *Glossina austeni* using the SIT. Earlier ecological work has been seriously hampered by the elusiveness of the fly and the problems associated with trapping insufficient numbers of flies in conventional tsetse traps (chapter 1 and 12) to make a sound analysis possible (Turner, 1984; Hall, 1986; Madubunyi, 1990). Trap inefficiency was most likely related to a poor trap entering response combined with a poor upward moving response (Hall, 1990). This upward movement, on which most tsetse traps rely to guide the flies into the non-return

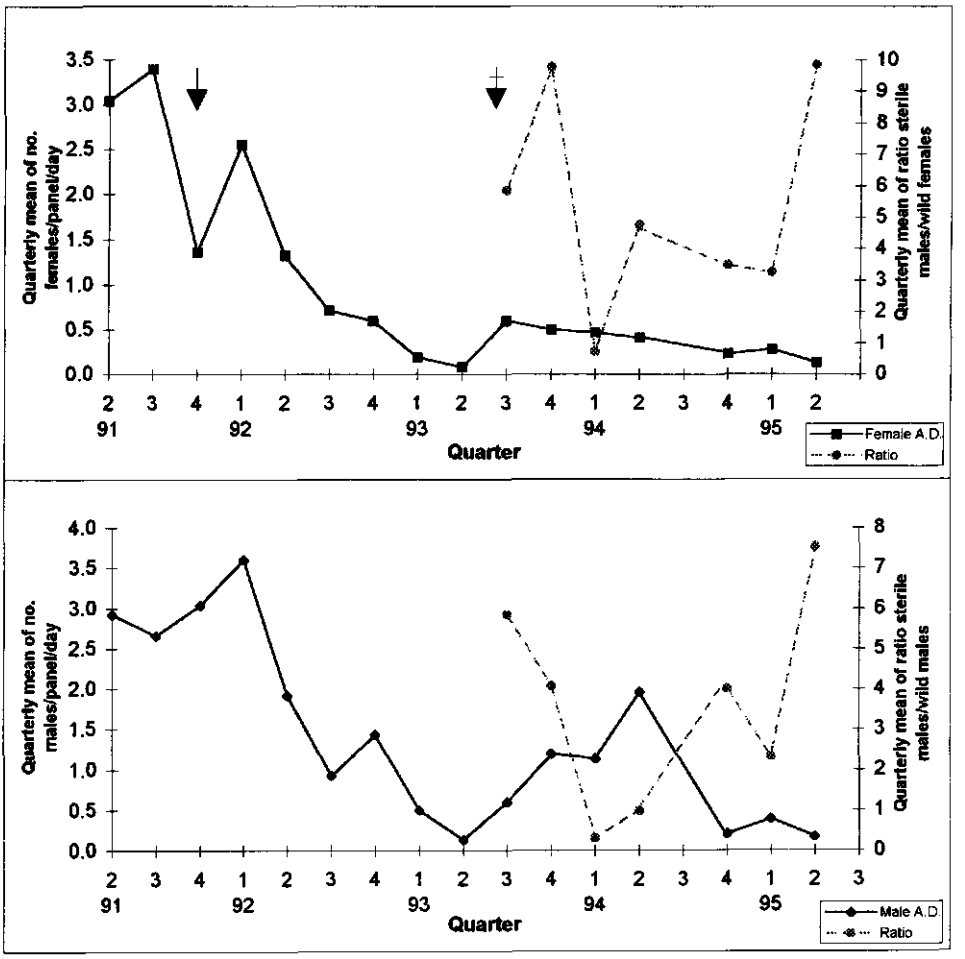

Fig. 1 Fluctuations in apparent density of female and male *G. austeni* in the north of the Jozani forest (JN) during prior suppression using IIS and during releases of sterile males and the ratio sterile males to wild females and wild males.

(→ : positioning of IIS; +→ : start releases)

cage, seems to be very weak in *G. austeni* (Takken, 1984). Lateral movements are apparently much stronger, which would explain the better catch efficiency of the sticky panels (Hall, 1986). Assessment of the efficiency of various models of sticky panels (described in chapter 13) demonstrated: (a) shape alone (3D versus MP and LP) did not play a key role in attractiveness for the fly, contrary to observations with other species (Vale, 1974), (b) the importance of white as a colour that attracts flies but did not induce a strong landing response, contrary to observations with *palpalis* species (Green, 1989) and (3) the significant impact of the type of sticky material used, indicating a potential attractiveness of some unidentified odours. Work is currently being conducted to identify potential olfactory attractants in cow - pig urine (Owaga, 1984), sebum of various wild hosts (Warnes, 1990) or ox-breath (Bursell, 1984; Takken, 1984). Based upon these results, the white monopanel (MP) (Vreysen *et al.*, 1992) and the Blue-White Legpanel (chapter 13) have been used in the SIT programme to monitor suppression of the wild fly population by means of IIS and the progress of the release programme. Although the use of sticky panels remains cumbersome, it has proven to be a reliable tool for entomological monitoring activities and initial concerns about possibilities of dissections of the female flies (Madubunyi, 1989) have been solved. A problem remains the significant undersampling of the female fly population, which should be addressed as females are needed to assess the impact of the release programmes (Van der Vloedt & Barnor, 1984). In case the apparent density of the fly population drops below the detectable level of the trapping device, the release of gamma sterilised females can be used to detect potential relic fly populations. The data presented in chapters 6 - 7 indicate that the mating behaviour of sterile females is not altered by the radiation treatment and female *G. austeni* remain receptive to mating until after the first ovulation.

Suppression of the original fly population was required (Cuisance *et al.*, 1980a) before releases could be initiated in view of the high initial fly densities in the Jozani forest (Vreysen, 1992). Application of residual or non-residual insecticides (Spielberger *et al.*, 1977; Molyneux *et al.*, 1978) was ruled out in view of environmental considerations in a forest reserve protecting endemic species. The lack of cattle in the forest did not permit the use of 'pour-on' insecticides (Höreth-Böntgen, 1992), and therefore, the only option was the deployment of insecticide impregnated screens (Laveissière *et al.*, 1981). The team of the UNDP/FAO Animal Disease Project, responsible for the prior suppression activities (UNDP/FAO, 1992), took the option of 'royal blue'

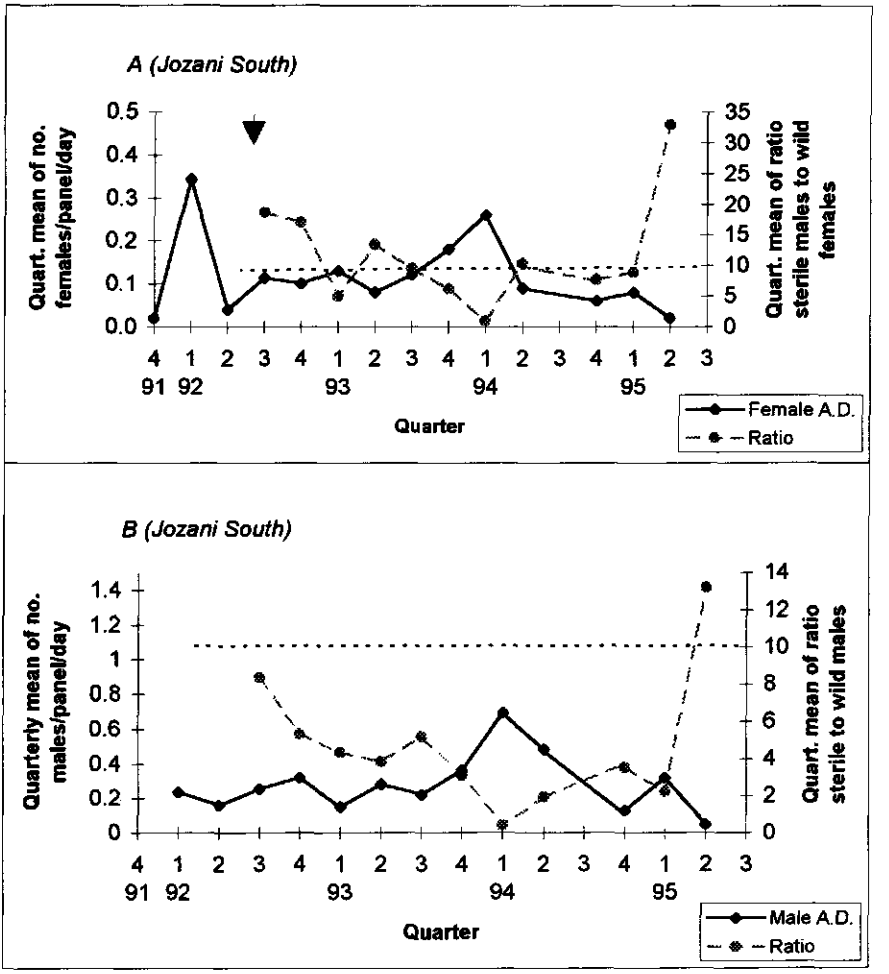

Fig. 2 Fluctuations in apparent density of female and male *G. austeni* in the south of the Jozani forest (JS) during releases of sterile males and the ratio sterile males to wild females and wild males. (→ : start releases).

as colour for the screens (Challier *et al.,* 1977; Green & Flint, 1986). This choice of 'royal blue' was somewhat arbitrary (UNDP/FAO, 1993) and influenced by the knowledge that black is completely unattractive for *G. austeni* (Turner, 1984; Hall, 1986) despite the fact that *G. austeni* belongs to the *morsitans* group (Vale *et al.,* 1985; Willemse, 1991). No direct evidence of the efficiency of 'royal blue' screens for attracting *G. austeni* was apparent at the moment of screen deployment (chapter 13). Recent experiments however, have shown equal efficiency of 'royal blue' and 'sky blue' sticky panels for catching sterile male *G. austeni* (Vreysen, unpublished). In view of the superior catch rate of blue-white legpanels, double cloth screens (blue on one side, white on the other) have been used in some areas of the forest, but the suppression effects as compared to all blue screens was never evaluated.

Suppression efforts proved to be very difficult in view of the dense forest habitat and the blue cloth screens (±1 m^2), impregnated with 0.2% alphacypermethrin (Fendona) or later with deltamethrin (Glossinex) had to be placed at densities of 30 and 70 screens per km^2 (Höreth-Böntgen, 1992, UNDP/FAO, 1993). Original apparent fly densities were on average 3.16 females/panel/day (range 2.86 - 3.40) and 2.83 males/panel/day (range 2.48 - 3.37) between April '91 and September '91 in the northern part of the Jozani (JN - Fig. 1, chapter 12). The male and female fly population density was reduced to 50 and 80 % respectively of its original value after 3 months but increased to 114% and 171% in the 4 th month, immediately before the annual wet season flooding of the forest floor. Fly population density was very low after the flooding (7% and 13% of original female and male population density) but increased rapidly to 25-37% and 44-76% for the female and male population respectively between month 10 and 12. Sixteen months after the initiation of the positioning of the screens, female and male apparent densities were as low as 11% and 3% respectively. The same trend was observed in the middle area of the Jozani forest (JM) with female and male apparent densities reduced to 36% and 10% after 16 months. This demonstrated that blue IIS can be deployed against *G. austeni* in dense forest habitats provided high densities of screens are used for relatively long periods. These observations are in contrast with the prior suppression activities in the savannah habitats of West Africa (Mérot *et al.,* 1984; Takken *et al.,* 1986) but rather similar to the observations in the dense rain forests of West and Central Africa (Laveissière *et al.*, 1986; Gouteux & Sinda, 1990; Lancien, 1991).

Releases of sterile males were initiated in the southern part of the Jozani forest (JS) in July '92 following 24 months of prior suppression and in JN in July '93 following 18 months of prior suppression. The fluctuations in apparent density of the native fly population during the releases is presented in Fig. 1 and Fig. 2. All insecticide impregnated screens were removed before initiation of the sterile male releases. Quality of the released male flies (described in chapter 14), based upon survival and dispersal data were comparable with those obtained for other tsetse species (Bourne, 1982; Cuisance & Itard, 1973; Wiilliamson *et al.*, 1983 b,c). After termination of the suppression activities with IIS, an apparent density (A.D.) of 0.02-0.34 females/panel/day and 0.02-0.24 males/panel/day were observed in the JS. During the period of release, the ratio of sterile males to wild females fluctuated between 5 and 15 except for the first quarter of 94 (ratio of 1:1) and

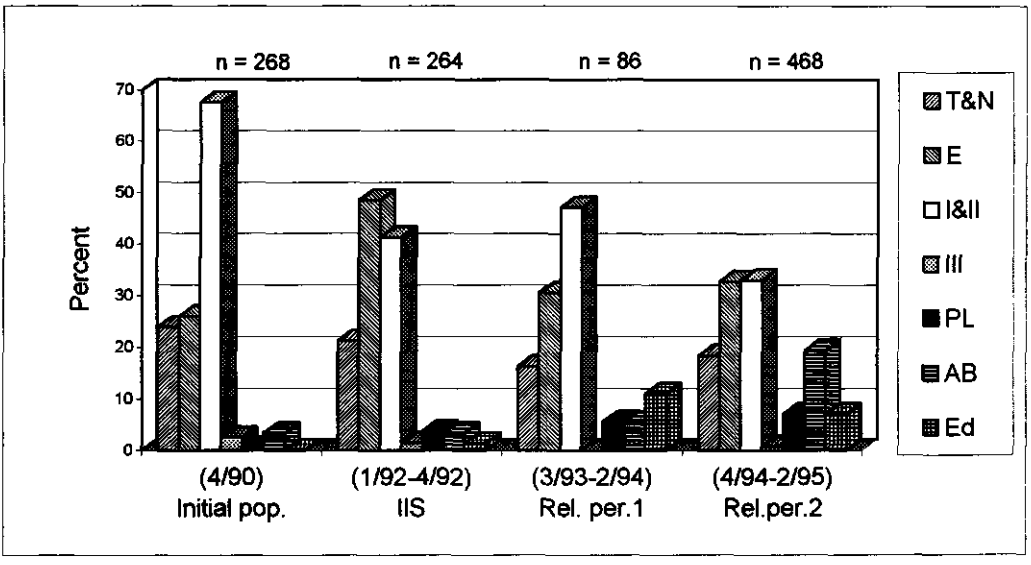

Fig. 3 The reproductive status of female *G. austeni*, sampled before control was initiated (Initial population), during the period of fly suppression with Insecticide Impregnated screens (IIS) and during the period of releases (Rel. period 1 (March '93 - February '94) - Rel. period 2 (April '94 - February '95). (T = teneral, N = nulliparous, E = egg, I,II,II = first, second and third instar larva, PL = post larviposition, AB = uterus empty due to abortion, Ed = egg in embryonic arrest).

the second quarter of 95 (ratio of 34:1). Female fly density did not increase after the initiation of the releases and remained below 0.26 females/panel/day. In JN, the native fly population was reduced to similar densities as in JS before the initiation of the releases. Ratios of sterile males to wild females did not exceed 10, but female A.D. remained below 0.5 females per panel per day. Male A.D. increased to 2 males/panel/day, but this was most likely an artefact due to the release of unmarked sterile males. Since the last quarter of '94, the male A.D. is likewise below 0.5 males/panel/day.

Accurate information on the impact of the releases on the female fly population can be provided by the reproductive status of the female flies (Van der Vloedt & Barnor, 1984) (Fig. 3). The initial female fly population, before the initiation of any control activity, was comprised of 23.9% 'Teneral and Nulliparous' females. Of the parous fly population, 26%, 67.6%, 2.5% of the females contained an egg, I - II and III instar *in utero* respectively. The natural abortion rate (November period) was established at 3.4%. No major changes in the nulliparous and parous proportion of the female population were observed during the period of IIS placement with the rate of abortion remaining at 3.4%. During the first release period, the proportion of females with viable larvae *in utero* remained the same (47%) but decreased to 33% during the second period of releases. The proportion of flies with PL, abortion or degenerating egg *in utero* fluctuated from 0.5 to 7.1%, 5.6% to 19.4% and from 11.1 to 7.1%. The rate of induced sterility for the two release periods was 16.7% and 26.5% for the parous female fly population.

The theoretical model of Knipling (1963) predicted that a ratio of 3 sterile male tsetse flies to 1 fertile would be sufficient to eradicate a stable tsetse population. This was contradicted during operational release programs in West Africa where a ratio of 10 sterile male flies to 1 fertile was required to obtain eradication (Politzar & Cuisance, 1984; Takken *et al.*, 1986; Oladunmade *et al.*, 1990). These ratios have not been reached in the eradication programme on Unguja and therefore, it is premature to expect eradication already. However, despite average ratios of 5.3-5.5 (sterile males to wild females) and 2.8-4.5 (sterile males to wild males), the native fly population remained very low and there was no recovery to original fly population densities.

The available data on ratios of sterile to fertile flies and the rate of induced sterility can be applied in the model of Fried (1971) to estimate the competitiveness of the sterile males and the predicted ratios required for the completion of the programme. In Fried's model, the ratio of S (Sterile) to N (Native) flies can be calculated by:

$$S/N = \frac{Ha - E}{E - Hs}$$

whereby Ha is the percent fertility resulting from a mating of a native male with native female, Hs is the fertility resulting from a mating of a sterile male with a fertile female and E is the expected fertility with the given ratio S/N. Assuming 95% and 5% fertility levels as values for Ha and Hs respectively, expected values for E are estimated between 20 - 31%, depending on the obtained ratios in the field. However, the fertility levels in the sampled female flies, based on the rate of induced sterility are 75-83%, indicating that a ratio of 19-21 sterile males to 1 fertile fly will be required.

As each species of tsetse fly most likely displays a different behaviour after irradiation and release in the field, it is difficult to make specific recommendations for release ratio's of *G. austeni* on the island of Unguja. The ratio's will most certainly be higher as predicted from the Knipling model. Whether one needs to go as high as 20 sterile males per 1 fertile male, will future work on Unguja show. As very limited information was available about the biology and ecology of *G. austeni,* and the species appears to behave quite different from other tsetse species, the merit of the first release experiments can be judged with optimism: considerable reduction of the fly population was achieved by the use of insecticide impregnated screens and the fly population remained under control despite sub optimal ratio's of sterile flies to wild flies indicating that a persistent release of higher numbers of sterile males will most certainly culminate in the extinction of the fly on the island of Unguja.

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Curriculum vitae

Mr. Marc J.B. Vreysen was born on 28 August 1958 in Turnhout, Belgium. He received his primary and secondary education in his home town, Mol. In 1976 he joined the Catholic University of Leuven where he obtained his degree in Zoology in 1981 (Licentiaat in de Wetenschappen). In 1986, he joined the Tropical Institute in Antwerp, Belgium, and obtained a postgraduate degree in Tropical Veterinary Medicine and Animal Science. From 1982 to 1984, he was associated with the Department of Entomology at the Catholic University of Leuven, conducting research on Vespidae. From 1984 to 1986 he worked as a lecturer in veterinary science in a rural development project for a Belgian Non-Governmental Organisation in the Republic of Zaire. In 1987, he joined the Food and Agricultural Organization of the United Nations and was assigned as Associate Professional Officer at the Entomology Unit of the IAEA's laboratory in Seibersdorf, Austria until 1990. He conducted research which constitutes the major part of this PhD. thesis. He was transferred to the Tsetse SIT eradication project in Zanzibar, United Republic of Tanzania in September 1990 where he works as an expert of the International Atomic Energy Agency until to date.