EFFECTS OF THE ENTOMOPATHOGENIC FUNGUS *METARHIZIUM ANISOPLIAE* ON *GLOSSINA FUSCIPES FUSCIPES* IN LAKE VICTORIA ISLAND

MARCO BRESE

A THESIS SUBMITTED IN PARTIAL FULFILMENT FOR THE DEGREE

OF A MASTER OF SCIENCE (M.SC.) IN ENTOMOLOGY OF

KENYATTA UNIVERSITY

Brese, Marco
Effects of the entomopathogenic

2000/258258

KENYATTA UNIVERSITY LIBRARY

01.02.2000

DECLARATIONS

This thesis is my original work and has not been presented for a degree in any other university or any other award.

Marco Brese 01.02.2000

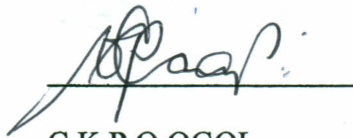
Marco Brese Date

I confirm that the work reported in this thesis was carried out by the candidate under my supervision.

Dr. Callistus K. P. O. Ogol

Department of Zoology

Kenyatta University, Nairobi



2/2/2000

C.K.P.O.OGOL Date

DEDICATION

Hiermit widme ich meine Arbeit meinem Vater Gerhard Brese and meiner Mutter Gudrun Brese.

Herewith, I dedicate my work to my father Gerhard Brese and my mother Gudrun Brese.

ACKNOWLEDGEMENT

Particularly, I am very grateful to my supervisor Dr. Callistus K. P. O. Ogol for his guidance in preparing this thesis. My appreciation goes to Dr. J. Perner and Dr. J. Schuhmacher for their assistance in statistical analysis. I am also very thankful to Dr. G. Jetschke and Dr. E. Finke for their assistance in the development of the computer model used.

The study was carried out within the Lethal Insect Technique (LIT) - project of the International Centre of Insect Physiology and Ecology (ICIPE). Therefore, I am particularly thankful to Dr. H. R. Herren, Director-In-General of ICIPE for the permission to use the necessary facilities and materials.

I'm indebted to Dr. N. K. Maniania, who provided and placed the contamination devices and the conidia of *M. anisopliae* on the study's disposal.

For valuable suggestions and comments to my work I am grateful to Prof. P. Langley, Dr. H. Mahamat, Dr. Copeland, Dr. D. J. Nadel, Dr. G. Tikubet, Dr. M. M. Mohammed-Ahmed, M. A. Okech, J. W. Wanyiri, P. Mbugi and P. Berry.

My sincere gratitude goes to J. O. Adino, P. Ogare, P. Khaemba, P. R. Akello, E. O. Ouma, A. T. L. Wanga, K. A. Ojuangu, K. O. Okoth, Z. Omondi and C. Meixner for their assistance.

ABSTRACT

Glossina spp. commonly known as tsetse flies are vectors of trypanosomes which can cause African human and animal trypanosomiases. The control of those diseases and their vector has shown some limitations. The 'Dissemination Technique' was considered to become a suitable method for tsetse control. It involves the contamination of flies with a lethal or sterilising agent and is based on the spread of the agent through natural contact events between tsetse flies. In the first part of the study, fluorescent pigment powder was used to detect whether there are regular contact events between specimens of *G. fuscipes fuscipes* in the field. The results show that males regularly contact other flies. The contact rate per male and day was positively linear correlated with the apparent population density. With a few exceptions, the contact events occurred regardless of sex or age. That suggests that morphological differences between males and females are too small to be detected from the males. It suggests further that male tsetse flies are attracted preliminary visually and that they may finally identify the sex and the willingness via contact. However, there were preferences for the youngest and oldest females and a discrimination against the youngest males. That could have been caused by olfactory chemicals or the behaviour of the flies. Male tenerals and the oldest males in wing-fray category 6 did not contact other flies while males in wing-fray categories 2 - 5 were found to be most sexually active. In the second part of the study, it was to determine whether a particular application of the 'Dissemination Technique' was an alternative to trapping

out. As a result, it was shown that Maniania's contamination devices, which were contaminated with dry conidia of the entomopathogenic fungus *Metarhizium anisopliae* and mounted on biconical traps, killed less flies than passing the CD's and hence, less flies than captured with the traps. That was because the infection rate of the CD was clearly less than 20 % and the number of infections through contact events between the flies too low to compensate that. Therefore, this particular application was found not to increase the efficiency of a trap. Hence, it is not an alternative to trapping.

TABLE OF CONTENTS

Title. i

Declarations ii

Dedication iii

Acknowledgement. iv

Abstract v

Table of contents vii

List of tables xii

List of figures xiv

List of plates xvi

CHAPTER ONE

GENERAL INTRODUCTION AND LITERATURE REVIEW . 1

1.1 Importance of the genera *Glossina* 1

1.2 Distribution 3

1.3 Identification and Taxonomy 4

1.4 Sexual behaviour 7

1.5 Life cycle 9

1.6 Tsetse flies as vectors of trypanosomes 10

1.7 Control of trypanosomiases 12

1.8 Control of tsetse flies 14

1.8.1 Bush clearing. 15

1.8.2 Game destruction 15

1.8.3 Insecticides 16

1.8.4 Trapping 19

1.8.5 Hand catching 20

1.8.6 'Sterile insect technique' (SIT) 21

1.8.7 Biological control 22

1.8.8 'Dissemination Technique' 23

1.8.9 Consequences of successful control 24

1.9 The tsetse fly *Glossina fuscipes fuscipes* Newstead 1910 . . 24

1.10 Entomopathogenic fungi 29

1.11 The entomopathogenic fungus *Metarhizium anisopliae* (Metchnikoff) Sorokin 32

1.12 Justification for the study and objectives 35

1.13 References cited 36

CHAPTER TWO

GENERAL MATERIALS AND METHODS 61

2.1 Study area 61

2.2 Climate 62

2.3 Study sites 62

2.3.1 Initial survey for suitable study sites 62

2.3.2 Chamaunga Islands 66

2.3.3 Wahondo Island 68

2.3.4 Kisui-thicket 68

2.4 References cited 70

CHAPTER THREE

EXPERIMENTS WITH FLUORESCENT PIGMENT POWDER

DETECTING CONTACT EVENTS BETWEEN TSETSE FLIES . 71

3.1 Introduction 71

3.2 Materials and methods 72

3.2.1 General laboratory experiments 72

3.2.2 Effect of marking 72

3.2.3 Transmission tests with fluorescent pigment powder . . 73

3.2.4 Determining the capture procedure for the field experiments . 75

3.2.5 Arrangement of the field experiments. 76

3.2.6 Marking and releasing of flies 77

3.2.7 Capture and treatment of flies 77

3.2.8 Determination and evaluation of contact events . . . 78

3.2.9 Determination of age 81

3.2.10 Preferences of contacting males 81

3.2.11 Sexual activity of males 82

3.2.12 Calculation of the average contact rate per male and day . . 82

3.2.13 Statistical analysis. 84

3.3 Results 85

3.3.1 The effect of marking 85

3.3.2 Transmission tests with fluorescent pigment powder . . 85

3.3.3 Determining the capture procedure for the field experiments . 86

3.3.4 Overall results of the field experiments 86

3.3.5 Preferences of contacting males 87

3.3.6 Sexual activity of males 88

3.3.7 Average contact rate per male and day 88

3.4. Discussion 89

3.4.1 Laboratory experiments 89

3.4.2 Preferences of contacting males 90

3.4.3 Sexual activity of males 94

3.4.4 Average contact rate per male and day 95

3.4.5 Potential of the 'Dissemination Technique' 96

3.5 References cited 107

CHAPTER FOUR

CONTROL EFFECT OF THE ENTOMOPATHOGENIC FUNGUS *METARHIZIUM ANISOPLIAE* ON A POPULATION OF *GLOSSINA FUSCIPES FUSCIPES* 111

4.1 Introduction 111

4.2 Materials and methods 112

4.2.1 Estimation of the control efficiency 112

4.2.2 Monitoring of the apparent population density 116

4.2.3 Ageing of flies 116

4.2.4 Mark-release-recapture study 117

4.2.5 Maniania's contamination device 118

4.2.6 Fungus application 120

4.2.7 Germination rate of the condia 121

4.2.8 Determination of the infection rate of the tsetse population . 121

4.2.9 Statistical analysis 122

4.3 Results 123

4.3.1 Apparent population density 123

4.3.2 Sex-ratio. 123

4.3.3 Age structure 123

4.3.4 Mark-release-recapture study 124

4.3.5 Germination rate of the conidia 124

4.3.6 Infection rate of the tsetse population. 124

4.3.7 Estimation of the control efficiency 125

4.4. Discussion 127

4.5 References cited 139

CHAPTER FIVE

GENERAL DISCUSSION AND RECOMMENDATIONS FOR

FUTURE RESEARCH WORK 143

LIST OF TABLES

Table	Title	Page
2.1	Results of the initial survey for suitable study sites . . .	65
3.1	Marking qualities on 4 subsequent days after male to female transmission of yellow fluorescent pigment powder by brief contact	103
3.2	Marking qualities on 4 subsequent days after male to female transmission of red fluorescent pigment powder by brief contact	103
3.3	Marking qualities on 4 subsequent days after male to female transmission of blue fluorescent pigment powder by brief contact	103
3.4	Marking qualities male to female transmission of yellow fluorescent pigment powder on 4 subsequent days by brief contact	104
3.5	Marking qualities on 4 subsequent days after transmission of yellow fluorescent pigment powder from 1 male to 10 females within2-3 hours by brief contact	104
3.6	Marking qualities of female to male transmission of yellow fluorescent pigment powder on 4 subsequent days by brief contact	104
3.7	Overall results of the field experiments on contact events	

	between tsetse flies using fluorescent pigment powder . .	105
3.8	P-values obtained when the pooled number of females and males respectively of experiments I, II, IV and V of each wing-fray category, which 'were contacted' and which 'were not contacted', was compared with the numbers of each other wing-fray category	106
3.9	P-values obtained when the pooled number of males and females of experiments I, II, IV and V of each wing-fray category, which 'were contacted' and which 'were not contacted', was compared with the pooled number of each other wing-fray category of the opposite sex	106
3.10	P-values obtained when the pooled number of males of experiments II, IV and V of each wing-fray category, which 'have contacted' and which 'have not contacted', was compared with the number of each other wing- fray category . . .	106
4.1	Estimation of the total number of flies on the northern Chamaunga Island prior to the exposure of the contaminated CD's using data on the mark-release-recapture study . .	138

LIST OF FIGURES

Fig. Title

Page

2.1 Average temperature (min., max.) and rainfall during the study period 63

2.2 Mean humidity and mean sunshine during the study period . 63

2.3 Map of the study area 67

3.1 Survival rates of flies due to the effect of red and yellow fluorescent pigment powder and handling 98

3.2 Survival rates of flies due to the effect of a) green and red acrylic colour and b) the combination of yellow fluorescent pigment powder and yellow acrylic colour and handling. 99

3.3 Percentage of males and females which 'were contacted' for each wing-fray category for experiments I, II, IV and V . . 100

3.4 Percentage of males which 'have contacted' for each wing- fray category for experiments II, IV and V 101

3.5 Correlation between the average contact rate per male and days and the apparent population density in ftd (flies per trap and day) for experiments I – IV 102

4.1 Sketch of the Maniania's contamination device 119

4.2 Apparent population density in ftd (flies per day and trap) a) of the northern Chamaunga Island and b) of the Kisui-thicket from March '98 to September '98 (— up to exposure of contaminated

CD's on the northern Chamaunga Island, – · – · – during exposure of the contaminated CD's on the northern Chamaunga Island) 133

4.3 Sex-ratio of the tsetse fly population on the northen Chamaunga Island and in the Kisui-thicket prior to and during the exposure of the contaminated CD's 134

4.4 Age structure of female tsetse population of the northern Chamaunga Island. 135

4.5 Age structure of the female tsetse population of the Kisui-thicket 135

4.6 Effect of the contaminated CD's at an infection rate of 100 % on a) only those females which had emerged up to exposure of the contaminated CD's and b) all females and the comparison with the observed field data 136

4.7 Maximum infection rate of the CD being a) 16.6 % when fitting the data on the observed final apparent population density and b) 10.4 % when fitting the data on the observed infection rate of the tsetse population 136

LIST OF PLATES

Plate Title

Page

2.1 Bird's-eye view of the Chamaunga Islands and a part of the Kisui thicket from the south 69

2.2 Wahondo Island showing the western lowland part which is characterised by big fig-trees with dense thicket vegetation . 69

4.1 Maniania's contamination device mounted on a biconical trap 119

CHAPTER ONE

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 Importance of the genera *Glossina*

Glossina spp. commonly known as tsetse flies are vectors of trypanosomes that cause African animal and human trypanosomiases. Little is known about the history of African human trypanosomiases, which is commonly known as human sleeping sickness. However, it was present for centuries in the Congo basin. The Arab historian Ibn Khaldun made the earliest report in the 14th century on the Niger at 5°W, whereby the King of Mali Mansa Djata was reported to have died of this disease in 1374 (Morris, 1963; McKelvey, 1973). The disease seemed to have spread throughout West Africa during the 18th century. In the early 18th century, the surgeon John Atkins reported in one of the first medical observations the 'Negro lethargy' while serving on slave ships between West Africa and West Indies. Today, it is known as the Gambian sleeping sickness. It is believed to have existed only in West Africa and in the Congo basin, whereby former isolated communities got increased contact through the colonial opening of the continent allowing the sickness to spread to East Africa. Other authors state that the trypanosomes were present in many other places, whereby only the absence of right number of people in the tsetse habits prevented further knowledge of the disease. The second type of human trypanosomiasis, the Rhodesian human sleeping sickness, was reported for the first time in 1896 in East Africa (McKelvey, 1973).

Gambian sleeping sickness is the more infective but less virulent endemic form and results in death mostly in two to three years if untreated. It is caused by *T. brucei gambiense*. Rhodesian sleeping sickness is the less infective but more virulent acute form and results in death in a few weeks or months if untreated. It is caused by *T. brucei rhodesiense* (Lambrecht, 1980a; Russell, 1993). Both are almost separately distributed, the former predominant in West and Central Africa and the latter in East and Southern Africa (Lambrecht, 1980a).

Disastrous epidemics were reported all over West, Central and East Africa. For example, between 1906 and 1920 the disease claimed in Congo hundreds of thousands of lives (Morris, 1963). Between 1905 and 1914 over 200,000 people, two thirds of the entire population, died in southern Busoga, Uganda, at the shores of Lake Victoria due to infections with *T. b. gambiense* (Lester, 1939; McKelvey, 1973). In Zaire, *T. b. rhodesiense* claimed about half a million victims in the 1970's (Cunningham, 1979). In 1995, an outbreak was reported in Zaire and Angola with few hundred thousand cases. Other countries that have experienced recent outbreaks of human sleeping sickness include Cameroon, Chad, Central African Republic and Côte d'Ivoire. In some districts of the western Upper Nile Region in southern Sudan, half of the human population has died within the last years due to a dramatic outbreak (ICIPE Annual Report 1995). African human trypanosomiasis was singled out as being one of the six major world human diseases that should be controlled (Anon., 1979) with over 100 million people being at potential risk. About 20,000 new infections are reported annually (ICIPE Annual Report 1994).

Animal trypanosomiasis or its vector, the tsetse flies are believed to be responsible for halting the southward and inland advances of Arabic peoples into Africa between 750 and 1500, by infecting their horses (Curtin *et all.*, 1978). Today, about 60 - 90 million cattle are at risk of trypanosomiasis, as well as tens of millions of goats, sheep, camels, horses and pigs (ICIPE Annual Report, 1994). The most important animal trypanosomiasis is Nagana, which affects cattle. About 7 million km^2 out of 10 million km^2 tsetse affected area in tropical Africa could be used for keeping cattle. Animal trypanosomiases with *Glossina* spp. as vector are caused by *T. vivax vivax, T. congolense congolense, T. brucei brucei, T. simiae, T. uniforme* and *T. suis* (Pollock, I). The infection is characterised by a progressive weakness, extreme loss of weight, anaemia, and if untreated, results in death. Animal trypanosomiases affect not only the supply of meat and milk but also crop farming and transport system since donkeys, camels and some cattle are still used in this way. Animal trypanosomiases are estimated to cause indirect economic losses of about US$ 5 billion yearly (ICIPE Annual Report, 1995). By 2025 Africa will have to feed 800 million additional people, that could be achieved only with a growth rate of food production of 2.75 % every year maintaining current levels. This figure is contrasting with a yearly growth of 1.7 % from 1961 to 1988 (ICIPE Annual Report, 1994).

1.2 Distribution

Tsetse flies occur in the African mainland and some closed islands (except Madagascar) between about 14°N and 29°S covering an area of about 10

million km^2 spanning over 38 sub-Saharan countries (Moloo, 1993). Tsetse flies have been reported up to an altitude of about 2,200 m above sea level (Tikubet and Gemetchu, 1984). However, the upper limit decreases generally with distance from the equator (Jordan, 1986). Exceptionally, a population of *G. tachinoides* in South Yemen (Carter, 1906) as well of *G. morsitans submorsitans* and *G. fuscipes fuscipes* in Southwestern Saudi Arabia have been reported, the latter occurring at 17°N, the northest latitude recorded for *Glossina* spp. (Elsen *et al.*, 1990).

Climate, feeding habits and vegetation determine the limits of tsetse distribution. The isotherm with the coldest monthly mean temperature of 21°C marks the northern boundary (Lambrecht, 1980a). Too dense or open areas are generally avoided by tsetse flies (Glasgow, 1963).

1.3 Identification and Taxonomy

The word 'tsetse' originated from the Tswana community in Botswana. Cumming established the name in English in 1850 through his popular book "Five years of a hunter's life in the interior of Africa" (McKelvey, 1973).

Tsetse flies are easy to identify. The wings are crossed shears-like. The ends of wings overtop the abdomen. Nevertheless, the identification of close relative species can be difficult. The size of flies vary between 6.5mm (*G. tachinoides*) and 14 mm (*G. nigrofusca*) (Glasgow 1963). There is no obvious sex-dimorphism. However, the characteristic hector and hypopygium at the hind-

sternites of males can easily distinguish the sex. In addition, the females are usually a bit bigger in average size (Pollock I).

Glossina spp. are dipterans and belong to the family Glossinidae. Buxton (1955) established the genera *Glossina* with *G. longipalpis* Wiedemann 1930 as generic type. Presently, some 23 species and 8 subspecies are known (Moloo, 1993) of which only six or seven are of major economic importance over extensive areas. These include *G. palpalis, G. tachinoides, G. fuscipes, G. morsitans, G. swynnertoni* and *G. pallidipes* (Jordan, 1994). *G. nashi* was in 1955 the last new species described and named (Pollock, I). Tsetse flies are classified into three groups, now recognised as sub-genera:

- the fusca group (sub-genera *Austenina*) including 15 species and sub-species,
- the palpalis group (sub-genera *Nemorpina*) including 9 species and sub-species and
- the morsitans group (sub-genera *Glossina*) including 7 species and sub-species (Moloo, 1993).

The fusca group also known as the forest species comprises *G. fusca, G. brevipalpis* and *G. longipennis.* Flies of this group are mainly found in primary rain forests but they can also be found in secondary forests, although *G. longipennis* is found in dry savannah woodland. Excluding *G. longipennis* and *G. brevipalpis,* they are of little economic significance, since they feed on man

only in exceptional cases. Furthermore, cattle are rarely where these species occur (Glasgow, 1963).

The palpalis group also known as the riverine species occupies mainly river systems and the shores of some great African Lakes, but not along river systems draining into the Indian Ocean (Jordan, 1986). In areas with long dry seasons, they occur just in evergreen groundwater-forests. *G. palpalis, G. fuscipes* and *G. tachinoides* are representative of this group (Glasgow, 1963). The palpalis group is known to be generally associated with the Gambian sleeping sickness (Willett, 1965).

The species of the morsitans group also known as the savannah species usually occur in dry woodland savannahs at the East African coast and in a belt around the tropical rainforests. The important species in this group are *G. morsitans, G. pallidipes, G. austeni* and *G. swynnertoni*. *G. austeni* is an exception of this group because it is found under evergreen conditions. However *G. austeni* never overlaps with species of palpalis group (Glasgow, 1963). If it is too hot and dry, the species of this group move into denser vegetation and if cooler and wetter they spread into the more open areas (Dransfield *et al.*, 1991). This group is known to be generally associated with the Rhodesian sleeping sickness, although there are several examples of converse associations with the palpalis group and the Gambian human sleeping sickness (Willett, 1965)

1.4 Sexual behaviour

The activity and aggressiveness of males in terms of mating behaviour increase with age in the first 1-2 weeks of the adult life (Nash, 1955; Nash *et al.*, 1971; Jaenson 1979). Males are capable of mating up to six times in the laboratory, whereby performance is improved through resting periods (Jordan, 1972). There is no evidence that the attractiveness of females to males diminishes with age (Langley, 1977).

Females become generally inseminated early in adult life (Buxton, 1955; Challier, 1982). In the laboratory, females are most receptive when 3-9 days old, which is species specific (Nash, 1955; Nash *et al.*, 1971; Rogers, 1972). The receptivity of females declines rapidly with the number of mating occasions and time after first mating (Tobe and Langley, 1978). It is known that refractory females can successfully resist copulation attempts. Females need to mate just once to be fertile for the rest of their lifetime by storing the sperm in the spermatheca (Pollock I). Sperm was shown to live in the spermathecae as long as 200 days after copulation (Glasgow, 1963). The duration of successful copulation can last from 14 to 220 min (Jordan, 1994).

The preliminary attraction of a male to a female fly is the first stage towards a successful copulation. However, there is no evidence for an olfactory attractant (Dean *et al.*, 1969; Turner, 1971) and for attraction by sound leading males to females (Langley, 1977). Instead it was shown in laboratory studies that the preliminary attraction is only visual (Wall, 1989). In addition, male/male

contacts were established, although very brief (Wall, 1989) suggesting morphological sex differences are too slight to be detected. These homosexual contacts although brief and always rejected by the target male are commonly known from laboratory studies (Coates and Langley, 1982). Therefore, Wall (1989) has shown that there is no olfactory male repellence. In contrast, Carlson and Schlein (1991) reported of volatile abstinons in the male cuticle preventing at least partly male/male contacts and homosexual behaviour. Furthermore, the contact events were shown to be caused not by random encounter. Colour seems to play no role for attraction (Wall, 1989).

Contact pheromones found in the wax layer of the female cuticle surface were found to cause sex recognition (Langley *et al.*, 1975; Carlson *et al.*, 1978). These pheromones do not cause an olfactory stimulation as shown in the laboratory and field, but males show copulatory behaviour when they get in contact with the pheromones (Hall, 1987; Wall, 1989). Langley *et al.* (1987) found chemoreceptors for contact pheromones on the tarsi and tibiae. The number and the duration of copulation attempts were shown to be dose dependent (Huyton *et al*, 1980b; Carlson *et al*, 1984). Contact pheromones are identified for several species including *G. morsitans morsitans* (Carlson *et al.*, 1978), *G. austeni* (Huyton *et al.*, 1980b), *G. pallidipes* (Carlson *et al.*, 1984) and *G. palpalis palpalis* (Offor *et al.*, 1981). The latter is a riverine species closely related to *G. fuscipes fuscipes.* The contact pheromones seem to be species specific (Huyton *et al.*,1980a). They are present throughout the life and increase with age (Huyton *et al.*,1980a). However, the copulatory behaviour of

a target-female is assumed to be even more important than the level of contact pheromone leading to a successful or unsuccessful copulation (Langley *et al.*, 1982; Carlson *et al.*, 1984). That is because females become generally inseminated early in adult life (Challier, 1982) and the receptivity of females declines rapidly with the number of mating occasions and the time after the first mating (Tobe and Langley, 1978).

Additionally, it has been proposed that specimens of *Glossina* spp. are identified by speed matching (by measuring the target's speed) meaning they would not contact non-tsetse flies with the same shape or size. This appears reasonable since tsetse flies fly up to 5 times faster than most other flies (Brady, 1991).

1.5 Life cycle

Tsetse flies are viviparous (Pollock, I). The same degree of viviparity within the class of insecta is otherwise only found in members of the very specialised group of the ectoparasiting *Pupipara (Diptera)* (Langley, 1977). Under ideal laboratory conditions, the time from the emergence of an adult female to the first larviposition is about 16 - 20 days. Further larvipositions follow at intervals of about 9-11 days. Only one larva grows at a time (Pollock I) nourishing from a liquid diet secretion produced by a milk gland in the uterus (Ma, 1974). Therefore, a fertile female gives birth to few (about 4-12) offspring in its entire life (Pollock, I).

The adult deposits the third instar at special shady, sun protected larvae sites. The larva moves slowly, does not feed after birth and is able to bury itself into a light ground to a depth of about 1-4 cm (Langley, 1977). The old larval cuticle forms the puparium, a protective case, within which the larva pupates (Zdarek and Denlinger, 1993). At an optimal temperature of about 24-25°C the period of pupal development ranges from about 30 days in *G. morsitans* to 49 days in *G. brevipalpis* (Zdarek and Denlinger, 1993). Generally, half of the whole tsetse population is present as pupae under the ground. The sex ratio of pupae is normally 1:1 (Pollock, I).

The emerging adult fly digs itself out from the soil. The newly emerged fly has a highly compressed abdomen and thorax (Zdarek and Denlinger, 1993). The increase in size from the newly emerged to the fully expanded tsetse is about 90% (Zdarek and Denlinger, 1992). Until the first blood meal, it has a characteristic soft body. This stage called teneral is only known for *Glossina* (Pollock I). The length of the life of tsetse depends on the species itself and the biotic and abiotic factors. The mean length may be only a few weeks or months with an extreme figure of about six month. Females live considerably longer than males (Buxton, 1955).

1.6 Tsetse flies as vectors of trypanosomes

Flies fed in laboratory are able to feed on almost all vertebrates. The natural hosts of tsetse flies include mammals, birds and even reptiles. The feeding habits can be divided into five main feeding patterns: feeding mainly on (a)

suids (b) suids and bovids (c) bovids (d) mammals other than suids and bovids and e) most available host and man (Challier, 1982). However, the feeding pattern are not absolute and differ a lot depending on locality or availability of host species (Lambrecht, 1980b). Generally avoided are duiker, Grant´s gazelle, impala, hartebeest, Zebra, baboon and dikdik (Muhigwa, 1998).

Flies pick up the trypanosomes while sucking blood from an infected mammal host. While undergoing some morphological and physiological changes the number of trypanosomes increases within 15-35 days. The life cycle of the *vivax*, *congolense* and *brucei* type of the trypanosomes differs in their sites of development in the fly including gut, salivary gland and proboscis. Thereafter the trypanosomes are infective. The fly remains infectious throughout its life. It spreads the infection by spittle during blood meals into new hosts. There is no transfer of the trypanosomes from the mother to the larva (Pollock, I). Tsetse flies are most susceptible to an infection when young (Lambrecht, 1980b). Baker (1958) reported a significant increase in lifespan of infected compared to non-infected flies. However, other laboratory results support the hypothesis that infected tsetse flies are less healthy than uninfected ones (Jenni *et al.*, 1980; Golder *et al.*, 1984).

Pathogenity of the trypanosomes varies with tsetse species, subspecies and even population (Harley and Wilson, 1968; Janssen and Wijers, 1974) and with the species of the donor-host (Ashcroft, 1959). Infection rate of the tsetse flies varies with the preferred host in the area, average age of the fly population

(Pollock, I) , tsetse species and sex (Moloo *et al.*, 1994; Moloo *et al.*, 1995), the species and race of trypanosomes (Moloo et al., 1994), the age of the single fly, the species of the donor-host, the environmental conditions, intercurrent infections and the composition of the trypanosoma population in the donor-host (Lambrecht , 1980b).

Trypanosomes are also transmitted mechanically. Tsetse flies and other biting flies such as *Stomoxys* and tabanids in Africa were found to do so. When they feed on an infected host and get interrupted, they may move and continue feeding on another animal and may inject some infected blood while still fresh from the first animal (Pollock, I). However, these other biting flies have no serious impact of African trypanosomiasis. Therefore, a positive linear relationship between tsetse density and trypanosomiasis has been shown (Roger, 1985).

1.7 Control of trypanosomiases

Although extensive research has been carried out during the last century, control of human sleeping sickness and animal trypanosomiasis still remains a problem. However, there are four basic ways of trypanosomiasis control: firstly to avoid tsetse-affected areas, secondly trypano-tolerance, thirdly control of the trypanosomes and fourthly control of its vector the tsetse flies.

Human settlement patterns are strongly influenced by tsetse flies showing an avoidance of affected areas. Within the class insecta, tsetse flies are assumed to

have the greatest impact on the human environment in tropical Africa (McKelvey, 1973).

In less trypanosomiases affected areas, that challenge has resulted in trypano-tolerant races of cattle (e.g. Muturu, NDama, Baoule), sheep and goats. Their tolerance developed due to exposure to low and medium trypanosomiasis challenge over several centuries especially in the more humid zones of West and Central Africa. However, they do not survive within areas with high risk of infection (Dransfield and Brightwell, 1992). Furthermore, they have stunted growth and cows give low amount of milk. Unsuccessful attempts were made to introduce them to East and Northeast Africa (Dotoum, 1979). But even in East Africa, there is now clear evidence of trypano-tolerance in zebu cattle (Dransfield and Brightwell, 1992).

Prophylactic measures involve regular injections with drugs with continuous effect. Therapeutic measures involve treatment just after infection. However, both methods are expensive. In addition, chemoprophylactical measures are just applicable in areas with a low risk of infection, and strong side effects arise usually during the therapy (Dransfield and Brightwell, 1992). Furthermore, the parasites develop resistance to these drugs (Nyeko *et al.*, 1988; Nyeko *et al.*, 1989). Probably no new drug will be developed in the near future since the economic return of these drugs is uneconomical to commercial companies (Dransfield and Brightwell, 1992).

1.8 Control of tsetse flies

The best option to control both human and animal trypanosomiasis is the management of its vector - the tsetse fly. Since the early 1900´s, attempts for tsetse control have continued (Dransfield and Brightwell, 1992).

Tsetse flies belong to the Holometabola. Therefore the eggs, larvae, the pupae and the adult could be the target of control. However, tsetse flies are viviparous (Pollock I). Furthermore, the larvae crawl only for a few minutes on the soil before burying itself into the ground in order to pupate immediately (Langley, 1977). In addition, pupae sites are difficult to find. Thus, only few attempts to control tsetse flies have targeted the pupae. For example, the pupae parasitoid *Syntomosphyrum* spp. were released in Malawi, Nigeria and Tanzania (Lamborn, 1925; Lloyd *et al.*, 1927; Nash, 1933). However, they achieved little in terms of control. The adult tsetse fly is therefore considered to be the only suitable stage for control attempts.

There are two possible options to deal with the vectors: eradication and control. Although eradication was achieved severally, it is very costly and re-invasion was a constant, widespread problem due to non-closed populations (Allsopp, 1984). In practice, control is considered today as the only realistic strategy over much of Africa (Dransfield and Brightwell, 1992). Therefore, several methods have been developed each with its own limitations.

1.8.1 Bush clearing

This method involves sheer bush clearing and partial bush clearing (only removing the woody vegetation) in order to destroy the resting sites of the flies (Turner and Brightwell, 1986). It can be considered as the first real approach to control tsetse flies either directly or indirectly as a result of human settlement. The method was extensively used for instance in the 1920's in Tanganyika (mainland of Tanzania). In the dry savannah zone of West Africa, the riverine vegetation was cut down to control species of the *palpalis* group (Pollock, III). However, as a direct control method, the maintenance of cleared or defoliated areas proved to be extremely costly. Furthermore, bush clearing is environmentally harmful and adaptation to new peridomestical habits has been reported (Okoth, 1986).

1.8.2 Game destruction

A disastrous rinderpest starting in 1890 in Egypt coming down through the lake great region of East Africa to South Africa, was finally controlled in 1898. This epidemic might have been the reason to apply this method, because in extensive areas of the Southern Africa not only millions of domestic and wild animals disappeared (McKelvey, 1973) but even the tsetse fly *G. morsitans*. Then a systematic shooting of tens of thousands of rhinoceroses, giraffes, lions, antelopes and gazelles was carried out between the 1930's and 1960's in Natal (South Africa), Rhodesia (Zimbabwe), Tanzania, Uganda, Zimbabwe and Sudan (Pollock, III; McKelvey, 1973). They were considered to be the natural hosts of *Glossina* spp. and natural reservoirs of trypanosomes. In some cases,

tsetse flies were completely eradicated as in Shinyanga / Tanzania. However, it was also observed that the flies simply switched to other hosts such as reptiles, smaller mammals or livestock and extended their range into uninfested areas (McKelvey, 1973). In addition, this measure is ecologically destructive and therefore undesirable.

1.8.3 Insecticides

Insecticides used for tsetse control include organochlorines such as DDT (dichlorodiphenyl trichloroethane), BHC (benzene hexachloride), dieldrin and endosulfan and synthetic pyrethroids such as deltamethrin, cypermethrin and permethrin (Alsopp, 1993).

Organochlorines are characteristically stable and relatively cheap and were used for ground spraying, because of their long persistence. DDT was determined in the time of World War II as a phenomenally effective insecticide. Later on, dieldrin and BHC appeared. All were applied widely against tsetse flies with often great success including complete eradication over huge areas (McKelvey, 1973). The later developed endosulfan is not very stable, but proved very effective for sequential aerial spraying (Alsopp, 1993). However, insecticides also harm non-target insect species including natural predators. For example it was shown that tsetse control measures using organochlorides caused high short-term mortality rates for insectivorous and non-insectivorous birds, mammals, fish, crustaceans and reptiles (Harrington and Bidlingmayer, 1958; Graham, 1964; Wilson, 1972). Moreover, residues of

DDT and dieldrin were detected in bird and crocodile eggs in affected areas (Tannock *et al.*, 1983; Wall and Langley, 1991).

Synthetic pyrethroids were tested in the last two decades (Spielberger *et al*, 1979), and are today the most used insecticides. They are very stable and have extremely high levels of toxicity, which makes them more poisonous for most insects than organochlorines (Pollock, III). On the other hand, they appear to be less harmful to the environment because they are only slightly toxic to mammals (Alsopp, 1993). However, synthetic pyrethroids were also found to seriously affect non-target species (Takken *et al.*, 1978). Moreover, their production and application is too costly especially for the developing countries (Bull, 1982).

Insecticide application includes selective ground spraying, sequential aerial spraying, insecticides treated livestock and insecticide impregnated targets.

Selective ground spraying means the spraying of residual deposits with a knapsack pressure sprayer selectively to tree trunks, lower branches of trees and other resting sites. It is highly labour-intensive and it becomes increasingly difficult to recruit casual labour for this demanding and unpleasant task (Allsopp, 1984).

Sequential aerial spraying is mostly applied from fixed wing aircraft (Pollock, III), whereby a certain unknown amount usually drifts out of the target area

(Allsopp, 1984). The first spray aims to kill all flies. Four to five subsequent sprays every ten days aim to kill the emerging flies before they can larviposit. This method aims at achieving a complete eradication of a fly focus. However, the technique is often not effective in very densely forested and mountainous areas (Allsopp, 1984).

Synthetic pyrethroids, mainly deltamethrin are applied to cattle by dipping, pour-on or spraying (Gao *et al.*, 1990; Okiria and Kalunda, 1994). In communities with dipping structure, it may be a good solution, but the procedure must be repeated every two weeks or even weekly in wet weather. This is difficult to achieve where large herds are involved. On the other hand, cattle must occur in a reasonable high density and should be fairly evenly distributed. Ticks are also controlled with such measures. However, since cattle develop a natural immunity to tick diseases when young, such operations are recommended not to be continuous, otherwise no immunity may develop and cattle can become susceptible to tick disease challenges (Dransfield and Brightwell, 1992).

Insecticide impregnated targets were firstly and successfully used in 1950´s by Rupp in Rwanda and Burundi against *G. fuscipes martinii* (Dransfield and Brightwell, 1992). In order to find a cheaper and more practical solution than the biconical trap, studies intensified in the 1970´s and ´80´s and came up with a series of insecticide-impregnated screens for the riverine species and later on for the savannah species (Torr, 1985). Several targets proved highly effective

(Vale *et al.*, 1986; Vale *et al.* 1988). However similar limitations as for traps arise (see 1.8.4).

1.8.4 Trapping

Studies for tsetse control through trapping were carried out widely in the 1920´s and ´30´s (Harris, 1932). This declined rapidly with the introduction of insecticides in the 1940´s. However, some alternative traps were developed in the 1950´s and ´60´s. But based on the earlier models, they were still large, difficult to transport and to set. In the late 1960´s the interest to control through trapping renewed again when it was apparent that the use of insecticides was not the best (Dransfield and Brightwell, 1992). Then the biconical trap (Challier and Laveissiére, 1973; Challier *et al.*, 1977) gave trapping as tsetse control strategy a big boost. Compared to earlier traps, it is small, cheap and very effective especially for the palpalis group. Out of this basic type, different modifications were developed such as the pyramidal trap (Gouteux and Lancien, 1986), monoconical trap (Lancien, 1981), F2 and F3 trap (Flint, 1985), vavoua trap (Laveissiére, 1990), monoscreen trap (Okoth, 1991) and the NGU trap series (Brightwell *et al.*, 1987; Brightwell *et al.*, 1991). This was in order to achieve a cheaper and more effective model and to find a good solution for the *morsitans* group.

Effective olfactory attractants such as acetone, 1-octen-3-ol and several phenols, cattle kairomones, can significantly increase the catch for species of the morsitans group (Dransfield and Brightwell, 1992). To date, among the

palpalis group, an effective attractant has only been determined for *G. tachinoides*. Riverine species are assumed to detect generally hosts by sight rather than by smell (Laveissiere *et al*., 1990).

Trapping can be very effective in tsetse control (Ryan *et al*., 1981; Opiyo, 1988). However, traps can not achieve eradication. Tsetse populations recover usually fast when control measures are interrupted. That is why this technique has to be used continuously. Often theft, natural damage and loss of traps due to bush-fire or seasonal floods as in case of the riverine species occur. Traps have to be served regularly because of rapid vegetation growth, which reduces the visibility. Moreover, traps are of limited duration due to the trap material (Laveissiere, 1990). In addition, for some tsetse species an efficient trap is not yet found (Dransfield and Brightwell, 1992).

1.8.5 Hand catching

This method was successfully applied in particular on riverine species including *G. palpalis* and *G. fuscipes fuscipes* in 1930´s and 1940´s in Nigeria, Kenya and Sudan (Glasgow and Duffy, 1951; McKelvey, 1973). Complete eradication could be achieved, for example at Kuja River in South Nyanza, Kenya (Glasgow and Duffy, 1947). However, increasing labour costs limit this method.

1.8.6 'Sterile Insect Technique' (SIT)

Von Borstel (1960) and Dame and Schmidt (1970) suggested this method. It involves the release of laboratory reared sterile male tsetse flies into the wild population and works by reducing the birth rate of the population. The method is based on the assumption that female tsetse flies mate only once in their lifetime. Furthermore, the laboratory-reared males must have generally the same fitness like the wild males (Vale *et al.*, 1976, Hall and Langley, 1989). According to Knipling (1963) a sterile: wild male ratio of 3:1 should result in eradication of tsetse within four complete generations, which corresponds to about one year. However, field trails have shown that a ratio of sterile to fertile wild males of about 10:1 is required to do so (Takken *et al.,* 1986).

Although a cost and manpower efficient mass rearing technique was developed (Nadel, pers.comm., 1998), the number of males available remains limited. That is why the method can work only at low-level infestations. It is only economical if complete eradication can be assured. Moreover, there are no differences in infection rates between sterile and fertile males and the temporary increase of infections in the treated area is possible (Moloo, 1984; Moloo, 1988). In addition, SIT requires expertise of the International Atomic Energy Agency (IAEA) due to the use of radioactive materials to sterilise the males by γ-irradiation.

1.8.7 Biological control

Jenkins (1964) listed 24 Hymenoptera species and 10 Diptera species to be parasitoids of tsetse pupae. Some work has been done on predators of tsetse pupae (Ford 1940, Potts 1950) and adult tsetse (Challier, 1982; Wangui 1996). However, no successful control attempt based on predators or parasitoids has been reported so far.

Some other parasitic organisms have been reported including the male sterilising DNA virus of *G. pallidipes* (Jaenson, 1978; Jura *et al.*, 1988; Odindo, 1988), rickettsia-like micro-organisms (Kokwaro *et al.*, 1990), nematode worms (Harley, 1966), bacteria (Kaaya and Darji, 1989; Kaaya and Okech, 1990b) and fungi (Kaaya, 1989). Antigens have also been reported to affect tsetse flies (Kaaya and Alemu, 1984). However, in most cases, application in control attempts were considered to be difficult, since the mentioned agents must be inoculated or taken orally by the fly with the exception of fungi.

The most promising biological control agents are the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae.* Laboratory and semi-field experiments have shown that the fungi cause high mortality rates of tsetse flies and that the infected fly is in the position to transmit the fungus to another fly (Kaaya and Okech, 1990a; Kaaya *et al.*, 1991; Maniania, 1998). However, so far no control attempt based on parasitic organisms has been reported.

1.8.8 'Dissemination technique'

The technique is based on passing a sterilising or lethal agent from contaminated released insects to other target-insects through contact events. This concept was firstly advocated by Masner *et al.* (1968) for *Pyrrhocoris apterus (Hemiptera: Pyrrhocorida)*. The technique has since been applied to various insect pests (McLaughlin *et al.* 1969; Shapas *et al.*, 1977; Jackson *et al.*, 1992; Vega *et al.*, 1995). Several studies were carried out considering the technique for tsetse control (Bursell, 1977; House, 1982; Langley, 1995; Maniania, 1998). Generally, there are two ways of contamination (Ethiopian Country Station, 1998). Firstly, captured wild tsetse flies are contaminated by passing through a contamination device (CD), which is mounted on a trap. The contaminated flies are then automatically released (House, 1982). The aim is therefore to increase considerably the efficiency of a trap. Secondly, contaminated mass reared tsetse flies can be released. Since females are needed to build up the colony, males are the objective of release. That approach may be favourable when dealing with tsetse species where there is no efficient trap and in areas with difficult access.

Several agents have been shown to affect tsetse flies including pathogens like bacteria (Kaaya & Darji, 1989), virus (Odindo, 1988) and fungi (Kaaya, 1989) and synthetical agents like antibiotics (Wetzel and Bauer, 1975), chitin synthesis inhibitor (Langley, 1995), antibodies (Kaaya & Alemu, 1984) and juvenile hormone mimics (Langley *et al.*, 1988). However, the mode of contamination of these agents limits their field application. Therefore, the most

promising agents have been identified as juvenile hormone mimics (Hargrove and Langley, 1993), chitin synthesis inhibitor (Langley, 1995) and entomopathogenic fungi including *Metarhizium anisopliae* and *Beauveria bassiana* (Kaaya, 1989; Maniania, 1998).

1.8.9 Consequences of successful control

Although human and animal trypanosomiases cause terrible suffering and economic losses, there are varying opinions considering the consequences of successful control or eradication. For example, Laveissiere *et al.* (1990) states that "finally, many scientists, economists and ecologists have recently begun to wonder if controlling tsetse flies is after all a good solution, since their disappearance would lead to the over-exploitation of the freed land, resulting in the destruction of the vegetation and bio-climatic changes and finally total desertification." Unwise use of new gained land may result in an even worse situation. Economically speaking, the benefits gained by tsetse control may be below the level of arising costs of restoration. Laveissiere *et al.* (1990) states further that "the challenge therefore is how to control the tsetse under these conditions."

1.9 The tsetse fly *Glossina fuscipes fuscipes* NEWSTEAD 1910

Glossina fuscipes fuscipes belongs to the palpalis group. According to Glasgow (1963), its length varies from 8 to 11 mm. *G. fuscipes* was once considered to be a sub-species of *G. palpalis*, but is now recognised as an independent

species including three sub-species: *G. f. fuscipes, G. f. martinii* and *G. f. quanzensis*.

G. fuscipes fuscipes is one of the main vectors of human sleeping sickness. For example, *G. fuscipes fuscipes* was the only vector of *T. gambiense*, which caused a disastrous outbreak of Gambian sleeping sickness between 1905 and 1914. That outbreak was responsible for the evacuation of the Basesse people from Sesse Islands in Lake Victoria and contributed to more than 200,000 deaths during an outbreak in southern Busoga, Uganda (Lester, 1939; McKelvey, 1973). Thereafter, *G. fuscipes fuscipes* was the only or at least the main vector during several outbreaks of Rhodesian sleeping sickness in Eastern Uganda and Western Kenya (Willett, 1965; Okoth and Kapaata, 1986). Similarly, outbreaks of Gambian sleeping sickness in Southern Sudan (Snow, 1984) and of animal trypanosomiases in large areas of East Africa were related to *Glossina fuscipes fuscipes* (Mwambu *et al*., 1971; Okuna and Mayende, 1981). Generally, *G. fuscipes fuscipes* is considered today as the main vector of the Gambian form of human sleeping sickness in Central and East Africa (Russell, 1993). It has risen as one of the six or seven tsetse species of major economic importance over extensive areas (Jordan, 1994).

G. fuscipes fuscipes is quite conservative in choosing habitats (Mulligan, 1970). It occurs usually close to water in linear forest vegetation along rivers and lakes, with humans and cattle near to them being at high risk. Mwangelwa *et al.* (1990) did not capture any fly more than 100 m from the shore at Rusinga

Island, Suba District (Kenya). However, the species can extend its normal riverine and lacustrine habitats. It was captured and found to breed even during the dry season 4 miles from any source of water and as far as 12 miles away from permanent water except small water holes (Chorley, 1944). It was found living and breeding in peridomestic ('around the home') situations mostly where coffee, banana, *Lantana camara* and other vegetation grew under larger trees like *Ficus*, *Mangifera* and *Cassia*. Furthermore, if its natural habitats and wild hosts are destroyed, *G. fuscipes fuscipes* will adapt itself completely to peridomestic situations (Okoth, 1986).

The present range includes the primary as well as the secondary forests and thickets in Congo, Gabon, Cameroon, Central Africa, Equatorial Guinea, Northern Angola, Western Tanzania, Western Kenya, Uganda, Western Rwanda, Western Burundi and small isolated belts south-west of Ethiopia, Sudan and Chad (Moloo, 1993; Pollock, I). It does not extend into river systems draining into the Indian Ocean. However, it has also been reported from Saudi Arabia (Elsen *et al.*, 1990).

Reports concerning the population dynamics do conflict. Okoth (1980) and Mwangelwa (1992) noted a drop during the wet season, while Harley (1965) and Van Vegten (1971) reported about equal abundance irrespective of season. Mwangelwa (1992) tried to explain this strong drop with a poor visibility of the flies to biconical trap rather than an actual decline of the population.

G. fuscipes fuscipes is a strictly diurnal subspecies (Harley, 1965). Activity rhythms showed unimodal patterns with main activity between 0800 hours and 1600 hours (Mohamed-Ahmed and Odulaja, 1997) with a rapid increase in morning and rapid fall in late afternoon (Harley, 1965). Mwangelwa *et al.* (1990), found activity peaks for females at 0900 hours and for males at 1000 hours. Mohamed-Ahmed and Odulaja (1997) reported activity peaks for females at 1300 hours and for males at 1100 hours. However, activity peaks were found to vary with site and season (Harley, 1965). Solar radiation, temperature and humidity were found to influence daily activity patterns (Mohamed-Ahmed and Odulaja, 1997; Mwangelwa *et al.* 1990).

The favoured hosts of *Glossina fuscipes fuscipes* are generally bushbuck (*Tragelaphus scriptus*) and monitor lizard (*Varanus niloticus niloticus* (Mulligan, 1970; Rogers, 1977; Okoth, 1980). Though, *G. fuscipes fuscipes* is an opportunistic feeder. An identification of 25,000 feeds from areas where a range of hosts were available had shown that 38 % of the flies fed on bovids (mainly bushbuck), 34 % on reptiles (mainly monitor lizard), 18 % on primates including man, 5 % on other mammals, 3 % on suids and 1 % on birds (Mulligan, 1970). However, along river forests in the Central African Republic, 87 % of blood meals were derived from wild ruminants (Gouteux *et al.*, 1994). Around Brazzaville *G. fuscipes fuscipes* was reported to feed as a 'village fly' nearly always on man (Pollock, II). In a area with the only potential mammals being *Hippopotamus,* cattle and human beings, 73 -98 % of the identified blood

meals of *G. fuscipes fuscipes* derived from the monitor lizard irrespective of season (Mohamed-Ahmed and Odulaja, 1997).

The most effective traps against *G. fuscipes fuscipes* are the biconical, pyramidal and monoscreen trap. However, different studies gave contradicting results (Dransfield and Brightwell, 1992). The monoscreen trap was reported in Uganda as more effective than the pyramidal, while less effective than biconical and pyramidal in Kenya (Dransfield and Brightwell, 1992). The pyramidal trap was reported to be more effective in Central Africa (Gouteux and LeGall, 1992) than the biconical trap and vice versa in Kenya (Mwangelwa *et al*., 1995). No odours are recommended for control purposes (Dransfield and Brightwell, 1992). Dransfield and Brightwell (1992) described the best trap position to the point: "For *G. fuscipes fuscipes*, optimal sites are along the border between two habitats of which at least one is wooded. The best sites are at the boundary between relict forest and plantations, forest and path, and forest and *Lantana* bush. Traps should also be set along rivers, and by swamps and water holes. They should contrast well with the background vegetation, and light should fall on the top of the trap." In fact, exact investigations have determined that best catches were obtained from the edge of a thicket or forest clump up to 5 m away (Mohammed-Ahmed and Wynolds, 1997)..

As a riverine species, typical problems arise when it comes to control. Non selective sprayings are limited due to an often closed canopy. They pollute water directly and are known to affect the aquatic fauna (Harrington and

Bidlingmayer, 1958). However, fish in particular is mostly the only or main source of protein for man in this areas. Furthermore, a lake or a river is usually the direct source of water for the adjoining human population. Much of the occupied area is usually difficult to access during heavy rain seasons to permit selective spraying or trapping. Since riverine species are less mobile then savannah species, a higher trap density and more release stations in case of the SIT are required. Rapid vegetation growth and therefore reduced visibility particularly in those habitats makes more frequent servicing of the traps necessary (Takken *et al.*, 1986; Laveissiere *et al.*, 1990).

1.10 Entomopathogenic fungi

The first experiment with an entomopathogenic fungus was conducted in 1835 by Agostino Bassi with *Beauveria bassiana* and the silkworm. The presence of entomopathogenic fungi in the tropics was reported since 1853. In 1874 Pasteur and LeConte suggested independently the use of fungi as pest control (Maniania, 1991). More than 400 species of entomopathogenic fungi have been found (Hall and Papierok, 1982), of which, only about 30 were intensively tested as mycopesticides for agriculture pests (Weiser, 1982; Ferron, 1981; Anderson *et al.*, 1988). However, so far no attempts were made to control insect vectors of human or animal diseases using entomopathogenic fungi. Since the late 1980's laboratory work on the potential of some species to control ticks and tsetse flies has been evaluated in the laboratory (Kaaya, 1989; Kaaya *et al.*, 1996).

Entomopathogenic fungi have advantages for insect control since they do not have to be inoculated or taken orally by the fly like bacteria or virus. Instead they pass through the cuticle, which makes the field application more practicable. They penetrate the cuticle of the living insect by a combination of mechanical pressure and enzymatic degradation by proteolytic enzymes and chitinase (Zacharuk, 1970; Lambiase and Yendol, 1977; St.Leger *et al*., 1986). The fungi produce a mass of hyphae inside the insect and mycotoxins which kill the host due to degeneration of the tissue caused by loss of structural integrity of membranes, followed by dehydration of cells. The end of the parasitic phase is marked by the death of the host. Thereafter, it grows saprophytically. Positive correlation between number of infective spores and mortality has been shown (Ferron, 1981; Weiser, 1982).

Entomopathogenic fungi are actually not of great importance in natural mass control of its hosts. This is because reproductive mycelia and conidiospores develop on the cuticle surface only in water saturated atmosphere (Ferron, 1981). They occur mostly as ordinary cases of fungal infection low in prevalence and constantly present in the host population. This is known as the enzootic form. Only in markedly damp years it is usually able to reduce the peak of outbreaks of its hosts due to epizootics. This was observed particularly in May beetle (*Melolontha melolontha*) infested areas (Schaerffenberg, 1964).

Although spore germination requires a comparable high relative humidity, infections were demonstrated irrespective of the surrounding low relative

humidity (Schaerffenberg, 1964; Ferron, 1981; Kaaya and Okech 1990a). Ferron (1981) attributed this to microclimate factors in the boundary layers of the host cuticle.

Methods of mass production have not changed very much since the pioneering effort by Krasilstschik in 1884 who produced *Metarhizium anisopliae* on beer malt and other ingredients (Weiser, 1982). However, the step from cultivation on solid artificial media to mass production was mostly unsuccessful and only deuteromycete fungi were produced on a larger scale (Weiser, 1982; Storey and Gardner, 1987). The virulence of the fungi depends on culture substrate and number of generations of artificial culture. Because the entomopathogenic fungi have a parasitic and a saprophytic phase, from time to time the fungi must be allowed to pass the parasitic phase (Schaerffenberg, 1964). As shown in most studies, persistence of entomopathogenic fungi declined dramatically in a few days if exposed to direct sunlight, whereby ultraviolet radiation was considered to be the major factor (Gardner *et al.*, 1977; Inglis *et al.*, 1993).

Several fungi have been reported to be associated with tsetse flies as natural pathogens. Nash (1933) suggested that a remarkable decrease of a population of *G. morsitans morsitans* in Tanganyika (Tanzanian mainland) was the effect of an entomopathogenic phycomycet, which caused black spots at sternites of flies. Up to 20 % of the catches were infected. After the rains, Lester (1934) reported of an unidentified fungal infection of 33% female *G. morsitans submorsitans* and 11% of female *G. tachinoides* in northern Nigeria. Hoof and

Henrad (1934) reported an extinction of a whole population of *G. palpalis* (probably *G. p. quanzenis*) at Leopoldville in Congo caused by a pathogenic fungus. A pathogenic fungus was found in the dry season on *G. brevipalpis* at the River Juba in Somalia (Moggridge, 1936). Vey (1971) discovered two entomopathogenic fungi (*Absidia repens, Penicillium lilacinum*) on pupae of *G. fusca* congolensis in Central African Republic. Turner and Makishe (1985) reported that in Zanzibar field collected pupae of *G. austeni* showed mortality rates up to 9 %, which may have been caused by a fungus.

1.11 The entomopathogenic fungus *Metarhizium anisopliae* (METCHNIKOFF) SOROKIN

M. anisopliae is a classical entomopathogenic fungus. The fungi causes mycoses known as green muscardines (Ferron, 1981). It is best known for its wide geographical distribution and broad host range (Hall and Papierok, 1982). It belongs to the class of Deuteromycetes (Hyphomycetes) (Maniania, 1991) and is a facultative pathogen (Fargues and Remaudiere, 1977). It is one of the few entomopathogenic fungi which is routinely mass-produced for control measures of different agricultural insect pests (Weiser, 1982). *M. anisopliae* is known to affect more than 200 insect species and is therefore considered as non-specific. However, numerous pathotypes were reported to be closely linked only to their respective hosts (Fargues and Remaudiere, 1977). Different strains were shown to have different natural virulence (Fargues and Remaudiere, 1977; Kaaya, 1989; Maniania, 1992).

M. anisopliae was reported to be infective at different stages of its *in-vitro* growth including as non-soaked and soaked conidia, pre-germinated conidia, blastospores and the mycelium itself (Hall, 1979; Maniania, 1991; Maniania *et al.*, 1993). It is applied in the field for example as a mycelial granular formulation (Wolfram *et al.*, 1990), spore formulation (Rath *et al.*, 1990) and dry conidia (Ferron, 1981).

The germination rate and infectivity of dry conidia of *M. anisopliae* declines only slightly for several months when stored under darkness at about 4-6°C and room temperature (Maniania, 1998 pers.comm.).

M. anisopliae has been used successfully and widely for biological control of agricultural pests (Anderson *et al.*, 1988), for example, against soil-inhabiting pests such as the black vine weevil, *Otiorhynchus sulcatus* (Wolfram *et al.*, 1990) and the rhinoceros beetle, *Oryctes rhinoceros*, a serious pest of coconut and oil palms (Munaan and Wikardi, 1986).

In the laboratory, *M. anisopliae* related mortality rates of contaminated adult flies were reported to be about 30 % (Poinar *et al.*, 1977) and 74 - 100 % (Kaaya, 1989; Kaaya and Okech, 1990a). Kaaya (1989) showed that the mortality rate increases directly with spore concentration. Males were reported to be more susceptible to the fungus than females (Kaaya, 1989; Kaaya and Okech, 1990a; ICIPE Annual Report, 1997). However Maniania (1994) stated that both male and female are equally susceptible.

There was no increase in abortion and pupal mortality produced by infected females (Kaaya, 1989). However, Kaaya and Okech (1990a) reported in contrast a significant higher mortality of those pupae produced by infected females.

Wilding (1969) showed that fungal infections could be transmitted from infected to healthy arthropods through physical contact. Tsetse flies contaminated with *M. anisopliae* successfully transmitted the infection to 45 % to 55 % of the 'recipient' flies when they remained together throughout the experiment at an original ratio of infected / non-infected flies of 1:1. Just 30 minutes exposure time to the contaminated flies caused fungus related mortality rates of 13 to 43 % of the recipients (Kaaya and Okech, 1990a). It was shown that one infected male can transmit a lethal amount of dry conidia of *M. anisopliae* to 70 % of the first, 65 % of the second, 60 % of the third and 20 % of the fourth female mates. Similarly, females transmitted to 80 %, 72.5 %, 57.5 and 45 % of the male mates (ICIPE Annual Report, 1997).

During laboratory studies, hyphae of *M. anisopliae* were shown in the fat body, muscles, haemolymph and gut walls of infected tsetse flies prior to death. No immune reactions such as phagocytosis or encapsulation in the infected tissues of the tsetse flies could be observed. Soon after death the fungus grew rapidly saprophytically through out the tissue and on the surface of the cuticle (Kaaya, 1989; Kaaya *et al.*, 1991)

1.12 Justification for the study and objectives

Glossina spp. are vectors of trypanosomes, the agents of African human and animal trypanosomiases. The control of the diseases and their vector has shown some limitations. There is a need to develop alternative methods, which are cost-effective, environmentally safe and adaptable particularly in rural areas. The 'Dissemination Technique' could become a very effective control method. It involves the application of lethal or sterilising agents and is based on contact between the specimens. Therefore, evidence has been reported that tsetse flies naturally get into contact for purposes of sex recognition. Potential agents were identified and tested successfully in the field. One of the promising agents is the entomopathogenic fungus *M. anisopliae*. The fungus can cause high mortality rates up to 100%. One of the recommended methods for tsetse control by the 'Dissemination Technique' is to contaminate captured wild tsetse flies by passing then through a contamination device (CD) contaminated with an agent and mounted on a trap in order to increase significantly the effect of a trap.

The objectives were:

a) to investigate the potential of the 'Dissemination Technique' for the control of *Glossina* spp.

b) to determine the control effect of the 'Dissemination Technique' on tsetse flies using Maniania's contamination device contaminated with dry conidia of *M. anisopliae* and mounted on biconial traps as an alternative to trapping.

1.13 References cited

- Allsopp, R. (1984). Control of tsetse flies (Diptera: Glossinidae) using insecticides: a review and future prospects. *Bulletin of Entomological Research* 74: 1 - 23
- Alsopp, N.J. (1993). *Training manual for tsetse control personnel Volume V*: Insecticides for tsetse and trypanosomiasis control using attraction bait techniques. FAO of the UN, Rome
- Anderson, T.E.; Roberts, D.W.; Sopes, R.S. (1988). Use of *Beauveria bassiana* for suppression of Colorado potato beetle in New York State (*Coleoptera: Chrysomelidae*). *Environmental Entomology* 17: 140-145
- Anon. (1979). *The African trypanosomiasis*, report of a joint WHO Experts committee and FAO Expert consultation. WHO, Geneva
- Ashcroft, M.T. (1959). The tinde experiment: a further study of the long-term cyclical transmission of *Trypanosoma rhodesiense. Ann. trop. Med . Parasit. 53: 137 - 146*
- Ashcroft, M.T.; Burtt, E.; Fairbairn, H. (1959). The experimental infection of some African wild animals with *T. rhodiense, T. brucei* and *T. congolense*. *Ann. trop. Med. Parasit*. 53: 147 – 161
- Baker, J.R. (1958). The longevity of trypanosome-infected tsetse flies. *E.A. Tryp. Res. Org. Rep. 1956-57*, Nairobi, E.A. High Commission, pp. 40 - 41
- Brady, J. (1991). Flying mate detection and chasing by tsetse flies (*Glossina*). *Physiological Entomology* 16, 153-161

- Brightwell, R.; Dransfield, R.D.; Kyorku, C.; Golder, T.K.; Tarimo, S.A.; Mungai, D. (1987). A new trap for *Glossina pallidipes*. *Tropical Pest Management* 33: 151-159
- Brightwell, R.; Dransfield, R.D.; Kyorku, C. (1991). Development of a low-cost tsetse trap and odour baits for *Glossina pallidipes* and *G.longipennis* in Kenya. *Medical and Veterinary Entomology* 5: 153 - 164
- Bull, D. (1982). *A growing problem: pesticides and the Third world poor*, Oxfam, Oxford, pp. 3-8
- Bursell, E. (1977). Chemosterilisation of tsetse flies using a pressurised metapa aerosol. *Trans. Rhod. Sci. Ass.* 58: 43 - 47
- Buxton, P.A. (1955). The natural history of tsetse flies. *Lond. Sch. Hyg. Trop. Med. Memoir* No.10, H.K.Lewis, London
- Carlson, D.A.; Langley, P.A.; Huyton, P. (1978). Sex pheromone of the tsetse fly: isolation, identification and synthesis of contact aphrodisiacs. *Science* 201, 750-753
- Carlson, D.A.; Nelson, D.R.; Langley, P.A.; Coates, T.W.; Davis, T.L.; Leegwater-Van der Linden, M.E. (1984). Contact sex pheromone in the tsetse fly *Glossina pallidipes* (Austen). Identification and Synthesis. *Journal of Chemical Ecology* 10(3): 429 - 450
- Carlson, D.A.; Schlein, Y. (1991). Unusual polymethyl alkenes in tsetse flies acting as abstinon in *Glossina morsitans*. *Journal of Chemical Ecology* 17 (2): 267 - 284

- Carter, R.M. (1906). Tsetse-fly in Arabia. *Brit. med. J.* 17/11/1906: 1393 - 1394
- Challier, A. ; Laveissiére, C. (1973). Un nouveau piége pour la capture des glossines: decription d'essais sur lé terrain. *Cahiers ORSTOM série Entomolgie médicale et Parasitologie* 11: 251-262
- Challier, A.; Eyraud, M.; Lafaye, A.; Laveissiére, C. (1977). Amélioration du rendement du piége biconique pour glossines (Diptera, Glossinidae) par l'emploi d'un cône inférieur bleu. *Cahiers ORSTOM, série Entomologie médicale et Parasitologie* 15: 283 - 286
- Challier, A. (1982). Mini-Review. The ecology of tsetse (*Glossina* spp.) (Diptera, Glossinidae): a review (1970 - 1981). *Insect Science and its Application* 3 (2): 97 - 143
- Chorley, T.W. (1944). *Glossina palpalis fuscipes* breeding away from water. *Proceed. of the Royal Entomological Society of London* A19: 1 - 4
- Coates, T.W.; Langley, P.A. (1982). The cause of mating abstention in male tsetse *flies Glossina morsitans. Physiological Entomology* 7: 235 - 242
- Cunningham, R.K. (1979). *The Tsetse Research Laboratory*, Langford. Bristol, United Kingdom.
- Curtin, P.; Feiermann, S.; Thompson, L; Vansina, J. (1978). *African History.* Longman, London.
- Dame, D.A.; Schmidt, C.H. (1970). The sterile-male technique against tsetse flies, *Glossina* spp. *Bulletin of the Entomological Society of America* 16: 24-30

- Dean G., Clements S., Paget J. (1969). Observations on sex attraction and mating behaviour of the tsetse fly, *Glossina morsitans orientalis*. *Bulletin of Entomological Research* 59, 355-365
- Dotoum, B. (1979). Note sur la situation sanitaire en matière des trypanosomiasis animales au Tchad au cours de la periode 1971 - 1975. - pp. 135 - 148 Int. Sci. Council Trypanosomiasis Res. Control. Fifteenth Meeting, Banjul, The Gambia, 1977. - 599 pp. Nairobi, *Scient. Publ. Div Eleza Services*. (Publication no.110).
- Dransfield, R.D.; Williams, B.G.; Brightwell, R. (1991). Control of Tsetse Flies and Trypanosomiasis: Myth or Reality? *Parasitology Today* 7 (10), 287-291
- Dransfield, R.D.; Brightwell, R. (1992). *Training for tsetse control personnel Vol. 4*: Use of attractive devices for tsetse survey and control. Food and Agriculture Organisation (FAO) of the UN, Rome, 1992
- Elsen, P.; Amoudi, M.A.; Leclercq, M. (1990). First record of *Glossina fuscipes fuscipes* Newstead, 1910 and *Glossina morsitans submorsitans* Newstead, 1910 in south-western Saudi Arabia. *Ann. Soc. Bèlg. Med. Trop.* 70: 281 - 287
- Ethiopian Country Station (1998). Community based *Glossina pallidipes* suppression model project in Southern Nations, Nationalities and People Regional State of Ethiopia, January 1998, a joint project executed by ECS, ESTC, SNNPRS, PPI and ICIPE

- Fargues, J.; Remaudiere, G. (1977). Consideration on the specificity of entomopathogenic fungi. *Mycopathologia* 62: 31-37
- Ferron, P. (1981). Pest control by the fungi *Beauveria* and *Metarhizium.* In „*Microbial control of pests and plant diseases 1970-1980*“ (H.D.Burges, Ed.), pp. 465-482. Academic Press, London.
- Flint, S. (1985). A comparison of various traps for *Glossina* spp. (Glossinidae) and other Diptera. *Bull. of Entomol. Research* 5: 529 – 534
- Ford, J. (1940). The action of predators on tsetse pupae. *Tsetse Research Report 1935 – 1938*, Dar es Salam, Govt. Printer, pp. 53 - 56
- Gao, M.K.; Mramba, F.; Bakuli, B.; Chakale, B. (1990). Efficacy of Decatix (deltamethrin 5%) cattle dip on tsetse flies at Mkwaja Ranch, Tanga Region. Tanzania Ministry of Agriculture and Livestock Development. *Research and Training Newsletter* 5: 3 - 5
- Gardner, W.A.; Sutton, R.M.; Noblet, R. (1977). Persistence of *Beauveria bassiana, Nomuraea rileyi* and *Nosema necatrix* on soybean foliage. *Environmental Entomology* 6: 616 - 618
- Glasgow, J.P.; Duffy, B.J. (1947). Extermination of *Glossina palpalis fuscipes*, Newstead, by hand catching. *Bulletin of Entomological Research* 38: 465-477
- Glasgow, J.P.; Duffy, B.J. (1951). Further observations on the extermination of *Glossina palpalis fuscipes* Newstead by hand catching. *Bulletin of Entomological Research* 42: 55 - 63

- Glasgow, J.P. (1963). International Series of Monographs on pure and applied Biogogy, Volume 20: *The Distribution and Abundance of Tsetse.* Pergamon Pres, Oxford-London-New York-Paris
- Golder, T.K.; Otieno, L.H.; Patel, N.Y.; Onyango, P. (1984). Increased sensivity to a natural pyrethrum extract of *Trypanosoma*-infected *Glossina morsitans. Acta Tropica* 41: 77 - 79
- Gouteux, J.P.; Lancien, J. (1986). Le piége pyramidal á tsé-tsé (Diptera: Glossinidae) pour la capture et la luttle. Essias comparatifs et description de nouveaux systémes de capture. *Trop. Medicine and Parasitology* 37: 61 - 66
- Gouteux, J.P.; LeGall, F. (1992). Piége bipyramidal á tsetse pour la protction de l'èlevage en Rép.Centraficaine. *World Animal Rev.* 70/71: 31 - 43
- Gouteux, J.P.; D'Amico, F.; Cuisance, D.; Blanc, F.; Demba, D.; Staak, C. ; Clause, P.H.; Kota Guinza, A.; LeGall, F. (1994). Feeding behaviour of *Glossina f. fuscipes* in two cattle breeding areas of the Central African Republic. *Vet. Res.* 25: 16 - 28
- Graham, P. (1964). Destruction of birds and other wild life, by dieldrex spraying against tsetse fly in Bechuanaland. *Arnoldia* 10(1): 1 - 4
- Hall, R.A. (1979). Pathogenity of *Verticillum lecanii* conidia and blastospores against aphid, *Macrosiphoniella sanborni. Entomophaga* 24: 191 - 198
- Hall, R.A.; Papierok B. (1982). Fungi as biological agents of arthropods of agriculture and medical importance. *Parasitology* 84: 205-240

- Hall, M.J.R. (1987). The orientation of males of *Glossina morsitans morsitans* Westwood (Diptera: Glossinidae) to pheromon-baited decoy 'females' in the field. *Bulletin of Entomological Research* 77, 487-495)
- Hall, M.J.R.; Langley, P.A. (1989). The responses of individual males in an isolated population of *Glossina morsitans morsitans* Westwood (Diptera: Glossinidae) to pheromone-baited decoy 'females'. *Bulletin of Entomological Research* 79: 319 - 334
- Hargrove, J.W.; Langley, P.A. (1993). A field trial of pyriproxyfen-treated targets as an alternative method for controlling tsetse (Diptera: Glossinidae). *Bulletin of Entomological Research* 83: 361 - 368
- Harley, J.M.B. (1965). Activity cycles of *Glossina pallidipes* Aust., *G. palpalis fuscipes* Newst. and *G. brevipalpis* Newst. *Bulletin of Entomological Research* 56: 141-160
- Harley, J.M.B. (1966). Studies on age and trypanosome infection rate in females of *Glossina pallidipes* Aust., *G. palpalis fuscipes* Newst. and *G. brevipalpis* Newst. in Uganda. *Bull. of Entomological Research* 57: 23 - 37
- Harley, J.M.B.; Wilson, A.J. (1968). Comparison between *Glossina morsitans, G. pallidipes* and *G. fuscipes* as vectors of trypanosomes of the *Trypanosoma congolense* group; the proportions infected experimentally and the numbers of infective organisms extruded during feeding. *Ann. trop. Med. Parasit.* 62: 178 - 187
- Harrington, R.W.; Bidlingmayer, W.L. (1958). Effects of dieldrin in fishes and invertebrates of a salt marsh. *J. Wildl. Management* 22(1): 76 - 82

- Harris, R.H.T.P. (1932). Some facts and figures regarding the attempted control of *Glossina pallidipes* in Zululand. *South African Journal of Science* 29: 495 – 507
- Hoof, L.Van; Henrard, C. (1934). La transmission cyclique des races résistantes de '*Trypanosoma gambiense*' par '*Glossina palpalis*'. *Ann. Soc. Belge. Med. Trop.* (Note complementaire) 14: 109 - 144
- House, A.P.R. (1982). Chemosterilisation of *Glossina morsitans morsitans* Westwood and *G.pallidipes* Austen (Diptera: Glossinidae) in the field. *Bulletin of Entomological Research* 72: 65 - 70
- Huyton, P.M.; Langley, P.A.; Carlson, D.A ; Coates, T.W. (1980a). The role of sex pheromones in initiation of copulatory behaviour by male tsetse flies, *Glossina morsitans morsitans*. *Physiological Entomology* 5, 243-252
- Huyton, P.M.; Langley, P.A.; Carlson, D.A.; Schwarz, M. (1980b). Specificity of contact sex pheromones in tsetse flies, *Glossina* spp. *Physiological Entomology* 5, 253-264
- ICIPE *Annual Report '94*
- ICIPE *Annual Report '95*
- ICIPE *Annual Report* '97
- Inglis, D.G.; Goettel, M.S.; Johnson, D.L. (1993). Persistence of entomopathogenic fungus, *Beauveria bassiana*, phylloplanes of crested wheatgrass and alfalfa. *Biological Control* 3: 258 - 270

- Jackson, D.M.; Brown, G.C.; Nordin, G.L.; Johnson, D.W. (1992). Autodissemination of baculovirus for management of tobacco budworms (Lepidoptera: Noctuidae) on tobacco. *J. Econ. Entomol.* 85: 710 - 719
- Jaenson, T.G.T. (1978). Virus-like rods associated with salivary gland hyperplasia in tsetse, *Glossina pallidipes. Transaction of the Royal Society of Tropical Medicine and Hygiene* 72: 234 - 238
- Jaenson, T.G.T. (1979). Mating behaviour of males of *Glossina pallidipes* Austen (Diptera: Glossinidae). *Bulletin of Entomological Research* 69, 573-588
- Janssen, J.A.H.A.; Wijers, D.J.B. (1974). *Trypanosoma siniae* at the Kenya coast. A correlation between virulence and the transmitting species of *Glossina. Ann. trop. Med. Parasit.* 68: 5 - 19
- Jenkins, D.W. (1964). Pathogens, parasites and predators of medically important arthropods. Annotated list and bibliography. *Bull. Wld. Hlth. Org. Suppl.* 30: 150
- Jenni, L.; Molyneux D.H.; Livesey J.L.; Galun, R. (1980). Feeding behaviour of tsetse flies infected with trypanosomes. *Nature, London* 283: 383 - 385
- Jordan, A.M. (1972). The insaminating potential of male *Glossina austeni* Newst. and *G. morsitans morsitans* Westw. (Dipt., Glossinidae). *Bulletin of Entomological Research* 62: 319 - 325
- Jordan, A.M. (1986). *Trypanosomiasis control and African rural development*. Longman, London

- Jordan, A.M. (1994). *A review of the tsetse research programme of ICIPE, 1971-94*
- Jura, W.G.Z.O.; Odhiambo, T.R.; Otieno, L.H.; Tabu, N.O. (1988). Gonadal lesions in virus-infected male and female tsetse, *Glossina pallidipes* (Diptera: Glossinidae). *Journal of Invertebrate Pathology* 52: 1-8
- Kaaya, G.P.; Alemu, P. (1984). Further observations on survival and fertility of *Glossina morsitans morsitans* maintained on immuized rabbits. *Insect Science and its Application* 5(5), 443-446
- Kaaya, G.P.; Darji, N. (1989). Mortality in adult tsetse, *Glossina morsitans morsitans*, caused by entomopathogenic bacteria. *Journal of Invertebrate Pathology* 54, 32-38
- Kaaya, G.P. (1989). *Glossina morsitans morsitans*: Mortalities caused in adults by experimental infection with entomopathogenic fungi. *Acta Tropica* 46: 107-114
- Kaaya, G.P.; Okech, M.A. (1990a). Horizontal transmission of mycotic infection in adults tsetse, *Glossina morsitans morsitans*. *Entomophaga* 35(4): 589-600
- Kaaya, G.P.; Okech, M.A. (1990b). Microorganisms associated with tsetse in nature: preliminary results on isolation, identification and pathogenity. *Insect Science and its Application* 11(3): 443-448
- Kaaya, G.P.; Kokwaro, E.D.; Murithi, J.K. (1991). Mortalities in adult *Glossina morsitans morsitans* experimentally-infected with the

entomogenous fungi, *Beauveria basiana* and *Metarhizium anisoplea. Discovery and Innovation* 3(3): 55-60

- Kaaya, G.P.; Mwangi, E.N.; Ouna, E.A. (1996). Prospects for biological control of livestock ticks, *Rhipicephalus appendiculatus* and *Amblyomma variegatum*, using the entomogenous fungi *Beauveria bassiana* and *Metarhizium anisopliae*. *Journal of Invertebrate Pathology* 67: 15-20
- Kenya Island Mission, Inc. (KIM) (1997). *Prospectus.*
- Knipling, E.F. (1963). Potential role of the sterility principle for tsetse fly eradication. 17pp. Geneva, Switzerland, *WHO / Vector Control 27*
- Kokwaro, E.D.; Nyindo M.; Chimtawi, M. (1990). Ultrastructural changes in salivary glands of tsetse, *Glossina morsitans morsitans*, infected with virus and rickettsia-like organisms. *Journal of Invertebrate Pathology* 56: 337 - 346
- Kuzoe, F.A.S. (1991). Perspectives in research on and control of African trypanosomiasis. *Annals of Trop. Medicine and Parasitology* 85 (1): 33 - 41
- Lambiase, J; Yendol, W.G. (1977). The fine structure of *Entomophthora apiculata* and its penetration of *Trichoplusia ni*. *Can. J. Microbiol.* 23: 452 - 464
- Lamborn, W.A. (1925). An attempt to control *Glossina morsitans* by means of *Syntomosphyrumn glossinae*, Waterston. *Bulletin of Entomological Research* 15: 303 - 309
- Lambrecht, F.L. (1980a). Paleoecology of tsetse flies and sleeping sickness in Africa. *Proc. of the American Philosophical Society* 124 (5): 367 - 385

- Lambrecht, F.L. (1980b). Ecological and physiological factors in the cyclic transmission of African trypanosomiasis. *Insect Science and its Application* 1: 47 - 54
- Lancien, J. (1981). Description du piege monoconique utilize pour l' elimination des glossines en Republique populaire du Congo. *Cah. ORSTOM. Ser. Entomol. Med. Parasitol.* XI, pp. 235 - 238
- Langley P.A., Pimley R.W., Carlson D.A. (1975). Sex recognition pheromone in tsetse fly *Glossina morsitans. Nature* 254, 51-53
- Langley, P.A. (1977). Physiology of tsetse flies (*Glossina* spp.) (*Diptera: Glossinidae)*: a review. *Bulletin of Entomologiacl Research* 67, 523-574
- Langley, P.A.; Coates, T.W.; Carlson, D.A. (1982). Sex recognition pheromone in the tsetse fly *Glossina pallidipes* Austen. *Experimentia* 38: 437 - 475
- Langley, P.A.; Huyton, P.M.; Carlson, D.A. (1987). Sex pheromone perception by males of the tsetse fly, *Glossina morsitans morsitans. Physiological Entomology* 12, 425-433
- Langley, P.A.; Felton, T.; Oouchi, H. (1988). Juvenile hormone mimics as effective sterilants for the tsetse fly *Glossina morsitans morsitans. Medical and Veterinary Entomology* 2, 29-35
- Langley, P.A. (1995). Evaluation of the chitin synthesis inhibitor triflumeron for controlling the tsetse *Glossina morsitans morsitans (*Diptera: Glossinidae*). Bulletin of Entomological Research* 85, 495-500

- Laveissiere, C. (1990). Sleeping sickness: peasants in charge of vector control. *International Scientific Council for Trypanosomiasis Research and Control. 20th meeting*, Mombasa. Kenya, 10-14th April 1989. Nairobi
- Laveissiere, C.; Eouzan, J.-P.; Grebaut, P.; Lemasson, J.-J. (1990). The control of riverine tsetse. *Insect Science and its Appl.* 11(3): 427 – 441
- Lester, H.M.O. (1934). Report on the tsetse investigation. *Rep. Med. Hlth. Serv. Nigeria 1933*: 74 - 83
- Lester, H.M.O. (1939). Certain aspects of Trypanosomiasis in some African dependences. *Trans. Roy. Soc. Trop. Med. Hyg.* 1: 11
- Lloyd, L.; Johnson, W.B.; Rawson, P.H. (1927). Experiments in the control of tsetse fly. *Bulletin of Entomological Research* 17: 423 - 455
- Ma, W.C. (1974). Secretory discharge and microflora of milk gland in tsetse flies. *Nature* 247 (5439): 301-303
- Maniania, N.K. (1991). Potential of some fungal pathogens for the control of pests in the tropics. *Insect Science and its Application* 12 (1,2,3): 63 - 70
- Maniania, N.K. (1992). Pathogenicity of entomogenous fungi (Hyphomycetes) to larvae of the stem borers, *Chilo partellus* Swinhoe and *Busseola fusca* Fuller. *Insect Science and its Application* 13 (5): 691-696
- Maniania, N.K.; Okello, R.O.; Oluoch, R.O.; Akinyi, J. (1993). Field evaluation of conidia and pre-germinated conidia of *Beauveria bassiana* (Bals.) Vuill. for the control of *Chilo partellus* (Swinhoe). *Journal of Plant Protection in the Tropics* 10 (2): 131 - 136

- Maniania, N.K. (1994). A laboratory technique for infecting adult tsetse with fungal pathogen. *Insect Science and its Application* 15 (4/5): 421 - 426
- Maniania N.K. (1998). A device for infecting adult tsetse flies, *Glossina* spp., with an entomopathogenic fungus in the field. *Biological Control* 11, 248-254
- Masner, P.; Slama, K.; Landa, V. (1968). Sexually spread insect sterility induced by the analogues of juvenile hormone. *Nature, London* 219: 395 – 396
- McKelvey, J.J.Jr. (1973). *Man against Tsetse. Struggle for Africa.* Ithaca, NY: Cornell University Press
- McLaughlin, R.E.; Cleveland, T.C.; Daum, R.J.; Bell, M.R. (1969). Development of the bait principle for boll weevil control. IV. Field tests with a bait containing a feeding stimulant and the sporozoans *Glugea gasti* and *Mattesia grandis. J. Invertebrate Pathology* 13: 429 - 441
- Moggridge, J.Y. (1936). Some observations on the seasonal spread of *Glossina pallidipes* in Italian Somaliland with notes on *G.brevipalpis* and *G.austeni. Bulletin of Entomological Research* 27: 449 - 466
- Mohamed-Ahmed, M.M.; Odulaja, A, (1997). Diel activity patterns and host preferences of *Glossina fuscipes fuscipes* (Diptera: Glossinidae) along the shores of Lake Victoria, Kenya. *Bulletin of Entomological Research* 87: 179-186

- Mohamed-Ahmed, M.M.; Wynolds, Y. (1997). Effects of vegetation and weather on trap catches of *Glossina fuscipes fuscipes* near Lake Victoria, Kenya. *Entomologia Experimentalis et Applicata* 85: 231 - 236
- Moloo, S.K. (1984). Infection rates in sterile males of *G. morsitans palpalis* and Fusca groups *Glossina* for pathogenic *Trypanosoma* spp. from East and West Africa. *Acta Tropica* 45(2): 145-152
- Moloo, S.K. (1988). Comparative study on the infection rates of different laboratory strains of *Glossina* spp. by *Trypanosoma congolense*. *Med. Vet. Entomol.* 2(3): 253-258
- Moloo, S.K. (1993). The distribution of *Glossina* species in Africa and their natural hosts. *Insect Science and its Application* 14(4): 511-527
- Moloo, S.K.; Zweygarth, E.; Sabwa, C.L. (1994). Comparative study on the susceptibility of different laboratory strains of *Glossina* species to *Trypanosoma simiae*. *Medical and Veterinary Entomology* 8(3): 225 – 230
- Moloo, S.K.; Zweygarth, E.; Sabwa, C.L. (1995). A comparison of susceptibility to stocks of *Trypanosoma simiae* of *Glossina pallidipes* originating from allopatric populations in Kenya. *Medical and Veterinary Entomology* 9(3): 224 - 228
- Morris, K.R.S. (1963). The movement of sleeping sickness across Central Africa. *Journal of Tropical Medicine and Hygiene* 66: 59 - 76
- Muhigwa, J.-B. B. (1998). *Glossina fuscipes fuscipes* Newstead 1910 (Diptera, Glossinidae): Colour preferences, host-finding strategies and responsiveness to baited traps. *PhD-thesis*, University of Khartoum

- Mulligan, H.W. (1970). *The African Trypanosomiasis*. Georg Allen & Unwin LTD, London, Boston, Sydney
- Munaan, A.; Wikardi, E.A. (1986). Towards the biological control of coconut insect pests in Indonesia, in „*Biological control in the tropics*"(Hussein, M.Y. and Ibrahim, A.G., Eds.) pp. 149-157
- Mwambu, P.M.; Mayende, J.S.P.; Masinde, A. (1971). *East African Trypanosomiasis Research Organisation Report, 1971*
- Mwangelwa, M.I.; Dransfield, R.D.; Otieno, L.H.; Mbata, K.J. (1990). Distribution and diel activity patterns of *Glossina fuscipes fuscipes* Newstead on Rusinga Island and Mainland in Mbita Point, Kenya. *Insect Science and its Application* 11(3): 315-321
- Mwangelwa, M.I. (1992). The ecology and vectorial capacity of *Glossina fuscipes fuscipes* on Rusinga Island along the shore of Lake Victoria. *PhD-thesis*. University of Zambia.
- Mwangelwa, M.I.; Dransfield, R.D.; Otieno, L.H.; Mbata, K.J. (1995). The response of *G. fuscipes fuscipes* Newstead to odour attractants and traps. *J. Afr. Zool.* 109: 23 - 30
- Nash T.A.M. (1933). The ecology of *Glossina morsitans* Westw., and two possible methods for ist destruction. *Bulletin of Entomological Research* 24: 107 - 157; 163 - 195
- Nash T.A.M. (1955). The fertilisation of *Glossina palpalis* in captivity. *Bulletin of Entomological Research* 46, 357-368

- Nash; T.A.M.; Jordan, A.M.; Trewern, M.A. (1971). Mass rearing of tsetse flies (*Glossina*: spp.): recent advances. pp. 99-110 in „*Sterility principle for insect control or eradication*“. Proceedings of a symposium, Athens, 1970. - 542 pp. Vienna, International Atomic Energy Agency. (Publ.no.STI/PUB/265)
- Nyeko, J.H.P.; Golder, T.K.; Otieno, L.H. (1988). Selection for drug resistance in *Trypanosoma congolense* during cyclic transmissions trough *Glossina morsitans morsitans* and drug treated rabbits. *Acta Trop.* 45, 21-26
- Nyeko, J.H.P.; Golder, T.K.; Otieno, L.H.; Ssenyonga, G.S.Z. (1989). *Trypanosoma congolense*: drug resistance during cyclical transmission in tsetse flies and syringe passage in mice. *Experimental Parasitology* 69 69: 357 - 362
- Odindo M.O. (1988). *Glossina pallidipes* Virus: its potential for use in biological control of tsetse. *Insect Science and its Application* 9(3), 399-403
- Offor, I.I.; Carlson, D.A.; Gadzama, N.M.; Bozimo, H.T. (1981). Sex recognition pheromone in the Weat African tsetse fly, *Glossina palpalis palpalis* (Robineau-Desvoidy). *Insect Science and its Appl.* 1(4): 417 - 420
- Okiria. R.; Kalunda, M. (1994). Knock down and survival of tsetse flies fed on cattle and pigs dipped in deltamethrin. *Annals of Tropical Medicine and Parasitology* 88 (1): 77 - 81
- Okoth, J.O. (1980). Observations on the composition of *Glossina* population at Lugala, South Busoga District, Uganda. *East African Medical Journal* 57(5): 332 - 335

- Okoth, J.O. (1986). Studies on the bio-ecology and taxonomy of *Glossina* with particular reference *to G. fuscipes fuscipes* Newstead. *M.Sc. Thesis*, University of Salford/UK
- Okoth, J.O.; Kapaata, R. (1986). Trypanosome infection rates in *Glossina fuscipes fuscipes* Newst. in the Busoga sleeping sickness focus, Uganda. *Annals of Tropical Medicine and Parasitology* 80(4): 459 - 461
- Okoth, J.O. (1991). Description of a mono-screen trap for *Glossina fuscipes fuscipes* Newstead in Uganda. *Annals of Tropical Medicine and Parasitology* 41: 517 - 519
- Okuna, N.M.; Mayende, J.S.P. (1981). The incidence of trypanosomiasis in domestic animals and their role in the sleeping sickness epidemic in Busoga. *ISCTRC, 18th Meeting* , Arusha, Publication No.112, p.163
- Opiyo, E.A. (1988). Tsetse control on Galana Ranch. Publication - *International Scientific Council for Trypanosomiasis Research and Control* 114: 434-437
- Poinar, G.O.Jr.; Van der Geest, L.; Helle, W.; Wassinsk, H. (1977). Pathology and nematode parasitism. In: M. Laird (ed.), *Tsetse: The Future for Biocontrol Methods in Integrated Control*. IDRC - 077e, Ottawa: 75-92
- Pollock, J.N. Ed. (I), (date unknown). *Training Manual for Tsetse control personnel*. Vol.1 Tsetse biology, systematics and distribution, techniques. FAO of the UN, Rome
- Pollock, J.N. Ed. (II), (date unknown). *Training Manual for Tsetse Control Personnel*. Vol.2 Ecology and behaviour of tsetse. FAO of the UN, Rome

- Pollock, J.N. Ed. (III), (date unknown). *Training Manual for Tsetse Control Personnel.* Vol.3 Control methods and side effects. FAO of the UN, Rome
- Potts, W.H. (1950). Tsetse Research, E.A. *Tsetse and Trypanosomiasis Research and Reclamation Organization Report 1950*, Nairobi, E.A. High Commission, pp. 11-33
- Rath, A.C.; Wright, P.J.; Yip, H.Y. (1990). Prospects for long term microbial control of the Tasmanian pasture pest complex. *Proceedings of the 5 th International Colloquium of Invertebrate Pathology and Microbial Control.* Adelaide, Australia, p. 339, August 20 - 24, 1990
- Rogers, A. (1972). Studies on the mating of *Glossina pallidipes* Austen. I. The age at mating. *Ann. trop. Med. Parasit.* 66: 515 - 523
- Rogers, D. (1977). Study of a natural population of *Glossina fuscipes fuscipes* Newst. and a model of fly movement. *J. Anim. Ecol.* 46: 309 - 330
- Rogers, D.J. (1985). Trypanosomiasis 'risk' or 'challenge': a review. *Acta Tropica* 42: 5 - 23
- Russell, R.C. (1993). Vector-borne diseases and their control. *The Medical Journal of Australia* 158: 681 - 690
- Ryan, L.; Molyneuy, D.H.; Kuzoe, F.A.S.; Baldry, D.A.T. (1981). Traps to control and estimate populations of *Glossina* species. *Tropenmedizinische Parasitologie* 32: 145 - 148
- Schaerffenberg, B. (1964). Biological and environmental conditions for the development of mycoses caused by *Beauveria* and *Metarhizium*. *Journal of Insect Pathology* 6: 8-20

- Shapas, T.J.; Burkholder, W.E.; Boush, G.M. (1977). Population suppression of *Trogoderma glabrum* by using pheromone luring for protozoan pathogen dissemination. *J. Econ. Entomol.* 70: 469 - 474
- Snow, W.F. (1984). Tsetse feeding habits in an area of endemic sleeping sickness in Southern Sudan. *Transactions Royal Soc. Trop. Med and Hygiene* 78(3): 413 - 414
- Spielberger, U.; Na'isa, B.K.; Koch, K.; Manno, A.; Skidmore, P.R.; Coutts, H.H. (1979). Field trials with the synthetic pyrethroids permethrin, cypermethrin and decamethrin against *Glossina (*Diptera: Glossinidae*)* in Nigeria. *Bulletin of Entomological Research* 69: 667-689
- St.Leger, R.J.; Charnley, A.K.; Cooper, R.M. (1986). Cuticle-degrading enzymes of entomopathogenic fungi: synthesis in culture on cuticle. *J. Invert. Pathol.* 48: 85 - 95
- Storey, G.K.; Gardner, W.A. (1987). Vertical movement of commercially formulated *Beauveria bassiana* conidia through four Georgia soil types. *Environmental Entomology* 16, 178-181
- Takken, W.; Balk, F.; Jansen, R.C.; Koeman, J.H. (1978). The experimental application of insecticides from a helicopter for the control of riverine populations of *Glossina tachinoides* in West Africa. VI: observations on side -effects. *PANS* 24: 455 - 466
- Takken, W.; Oladunmade, M.A.; Dengwat, L.; Feldman, H.U., Onah, J.A., Tenabe, S.O.; Hamann, H.J. (1986). The eradication of *Glossina palpalis palpalis* (Robineau-Desvoidy) (Diptera: Glossinidae) using traps,

insecticide-impregnated targets and the sterile insect technique in central Nigeria. *Bulletin of Entomological Research* 76: 275-286

- Tannock, J.; Howells, W.W.; Phelps, R.J. (1983). Chlorinated hydrocarbon pesticide residues in eggs of some birds in Zimbabwe. *Environmental Pollution* 5: 147 - 155
- Terry, R.J. (1976). Innate resistance to trypanosome infections. In „*Biology of the Kinetoplastida*", (Ed. by Lumsden and Evans), pp. 477 - 492. Academic Press, London
- Tikubet, G.; Gemetchu, T. (1984). Latitudinal distribution of tsetse in the Finchaa river valley (Western part of Ethiopia). *Insect Science and its Application* 5 (5): 389 - 395
- Tobe S.S., Langley P.A. (1978). Reproductive physiology of *Glossina. A.Rev.Ent.* 23, 283-307
- Torr, S.J. (1985). The susceptibility of *Glossina pallidipes* Austen (Diptera: Glossinidae) to insecticide deposites on targets. *Bulletin of Entomological Research* 78: 31 - 49
- Turner D.A. (1971). Olfactory perception of live hosts and CO2 by the tsetse fly, *Glossina morsitans orientalis. Bulletin of Entomological Research* 61, 75-96
- Turner, D.A.; Makishe, F. (1985). The distribution of *Glossina austeni* on Zanzibar. *International Scientific Council for Trypanosomiasis Research and Control*, 18th Meeting Harare (Zimbabwe) 1985: 263 - 271

- Turner, D.A.; Brightwell, R. (1986). An evaluation of a sequential aerial spraying operation against *Glossina pallidipes* Austen (Diptera: Glossinidae) in the Lambwe Valley of Kenya: Aspects of post-spray recovery and evidence of natural population regulation. *Bulletin of Entomological Research* 76: 331-349
- Vale, G.A.; Hargrove, J.W.; Jordan, A.M.; Langley, P.A.; Mews, A.R. (1976). Survival and behaviour of tsetse flies (Diptera, Glossinidae) released in the field: a comparison between wild flies and animal-fed and *in vitro*-fed laboratory-reared flies. *Bulletin of Entomological Research* 66: 731 - 744
- Vale G.A., Hargrove J.W., Cockbill G.F. (1986). Field trials of baits to control populations of *Glossina morsitans morsitans* and *G. pallidipes* Austen (Diptera: Glossinidae). *Bull. of Entomological Research* 76, 179-193
- Vale, G.A.; Lovemore, D.F.; Flint, S.; Co, G.F. (1988). Odour-baited targets to control flies. *Glossina* spp. (Diptera: Glossinidae), Zimbabwe. *Bulletin of Entomological Research* 78: 31 - 49
- Vanden Berghe, L. (1956). Aspect particular de la trypanosomiasis, maladie des pêcheurs, au Lac Tumba (Congo Belge.). *Ann. Soc. Bel. Med. trop*. 36: 185 - 190
- Van Vegten, J.A. (1971). Choice of food of *Glossina fuscipes fuscipes* living in thickets away from open water in south eastern Uganda. *International Scientific Council for Trypanosomiasis research. 13th meeting*, Lagos, 7-11.09.71, pp. 205-206. OAU/Scientific and Technical Reseach Commission (Publ.105)

- Vega, E.F.; Dow, F.P.; Bartelt, J.R. (1995). Dissemination of microbial agents using an autoinoculating device and several insect species as vector. *Biological Control* 5: 545 – 552
- Vey, A. (1971). Recherches sur les champignons pathogénes pour les glossines. Etudes sur *Glossina fusca congolensis* Newst. Et Evans en République Centrafricaine. *Rev. Elev. Méd. Vét. Pays Trop.* 24: 577 - 579
- Von Borstel, R.C. (1960). Population control by release of irradiated males. *Science* 131: 878-882
- Wall, R. (1989). The roles of vision and olfaction in mate location by the tsetse fly *Glossina morsitans morsitans. Medical and Veterinary Entomology* 3: 147 - 152
- Wall R., Langley P. (1991). From behaviour to control: the development of trap and target techniques for tsetse fly population management. *Agricultural Zoology Reviews* 4, 137-159
- Wangui, J.W. (1996). A study of the predators of the tsetse fly *Glossina austeni* Newstead (Diptera: Glossinidae) at Muhaka Forest, south coast Kenya. *M.Sc. - thesis*, Kenyatta University, unpublished
- Weiser, J. (1982). Persistence of Fungal Insecticides - Influence of environmental factors and present and future applications: In „*Microbial and viral pesticides*“, pp.231 - 257, ed. E.Kurstak, New York: Marcel Dekker, Inc.

- Wetzel, H.W.; Bauer, B. (1975). Influence of antibiotics on *Glossina morsitans* Westwood. pp. 501-505 in „*Sterility principle for insect control*“ 1974.-622pp. Vienna, IAEA/FAO: (Publ.no.STI/PUB/377)
- Wilding, N. ((1969). Effect of humidity on the sporulation of *Entomophthora aphids* and *E. thaxteriana*. *Trans. Br. mycol. Soc.* 53: 126-130
- Willett, K.C. (1965). Some observations on the recent epidemiology of sleeping sickness in Nyanza Region, Kenya, and ist relation to the general epidemiology of gambian and rhodesian sleeping sickness in Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 59 (4): 374 - 394
- Wilson, V.J. (1972). Observations on the effect of dieldrin on wildlife during tsetse fly *Glossina morsitans* control operations in eastern Zambia. *Arnoldia* 34(5): 1 - 12
- Wolfram, A.; Jurgen, H.; Paul, R.; Klaus, S. (1990). Production of mycelial granules of the entomopathogenic fungus *Metarhizium anisopliae* for biological control of soil pests. *Proceedings of the Fifth International Colloquium on Invertebrate Pathology and Microbial Control*, Society for Invertebrate Pathology, Adelaide, Australia, pp. 2-5
- Zacharuk, R.Y. (1970). Fine structure of the fungus *Metarhizium anisopliae* infecting three species of larval Elateridae (Coleopera). I. Dormant and Germinating Conidia. *Journal of Invertebrate Pathology* 15: 63 - 80

- Zdárek, J.; Denlinger, D.L. (1992). Eclosion behaviour in tsetse: extrication from the puparium and expansion of the adult. *Journal of Insect Behaviour* 5: 657 - 668
- Zdárek, J.; Denlinger, D.L. (1993). Metamorphosis behaviour and regulation in tsetse flies (*Glossina* spp.) (Diptera: Glossinidae): a review. *Bulletin of Entomological Research* 83: 447 - 461

CHAPTER TWO

GENERAL MATERIALS AND METHODS

2.1 Study area

The study area was located in Suba and Siaya Districts, Nyanza Province of Western Kenya in Lake Victoria (Fig. 4). The field experiments were based at Mbita Point Field Station (MPFS) of the International Insect Centre for Physiology and Ecology (ICIPE) which is situated at 00°26' south and 034°12' east. It is located on the foot of Gembe Hills and in front of the 43 km^2 Rusinga Island which is joined to the mainland by a causeway of about 100 m length. The vegetation around Mbita Point has been greatly influenced by human activity due to a remarkable increase of the human population from 1980 to a few thousand people today. The uplands, formerly covered with trees, shrubs and tall grass have been indiscriminately burned, deforested and overgrazed resulting in extreme depletion of soils and now only excessive bushland exists culminating in semi-desert conditions (KIM, 1997). The lowland is scattered and relatively densely inhabited and covered with grassland and scattered shrubs. A discontinuous thicket strip occurs along the shoreline with a width mostly not exceeding 100 m. The lake side is characterised by a few islands of different sizes. The inhabited islands have the same environmental situation as on the mainland, while the uninhabited islands show either a rocky or bushy environment. The only tsetse species occurring in the area is *G. fuscipes fuscipes* inhabiting only the thicket strip along the shore of the mainland and the islands (Mwangelwa *et al.*, 1990).

2.2 Climate

According to the National Atlas of Kenya (1970), the study area receives a mean annual rainfall of between 760 – 1015 mm and has a minimum and maximum temperature of between 14 – 18 °C and 30 - 40 °C respectively. Generally, there is rainfall throughout the year with peaks from March to May and in November. The lowest rainfall is commonly in February and July to September. Temperatures fluctuate only slightly without clear seasonal changes.

During the study period, temperature were recorded daily with a min.-max.-thermometer (min. at 0900 hours, max. at 1500 hours), rainfall daily at 0900 hours using a raingauge, sunhours daily using a Compel Sunshine Recorder and humidity daily at 1500 hours with a dry/wet bulb-thermometer all at the weather-station of MPFS (Fig. 2.1, 2.2).

2.3 Study sites

2.3.1 Initial survey for suitable study sites

In order to determine suitable sites for the field experiments requiring closed populations of tsetse flies, an initial survey was carried out in January and February 1998 on small islands around Mbita Point. These islands included Chamaunga Islands, Sentinel Island, Wahondo Island, America Island, Bridge

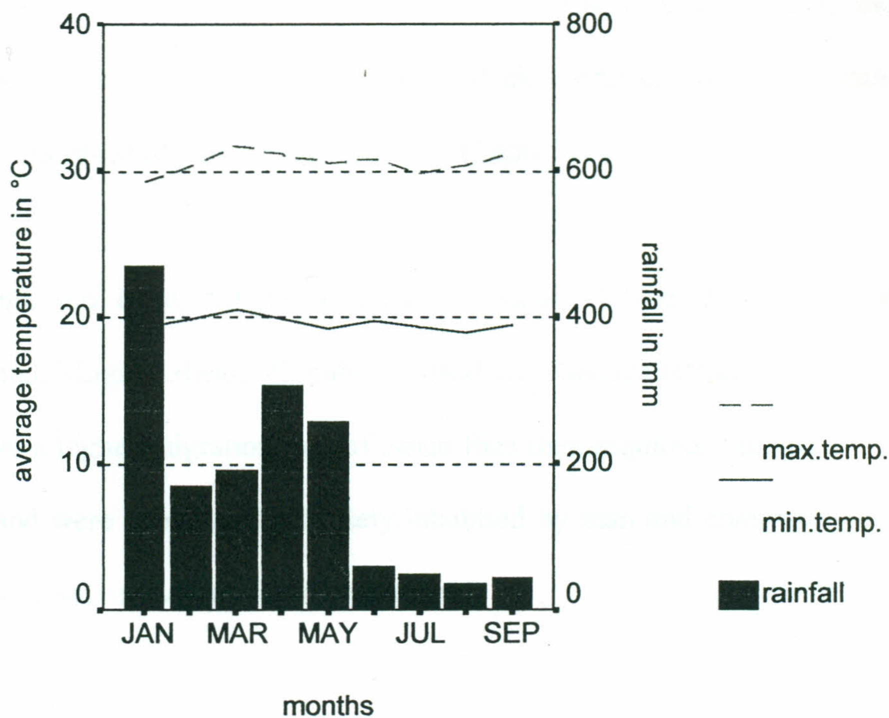


Figure 2.1 Average temperature (min., max.) and rainfall during the study period.

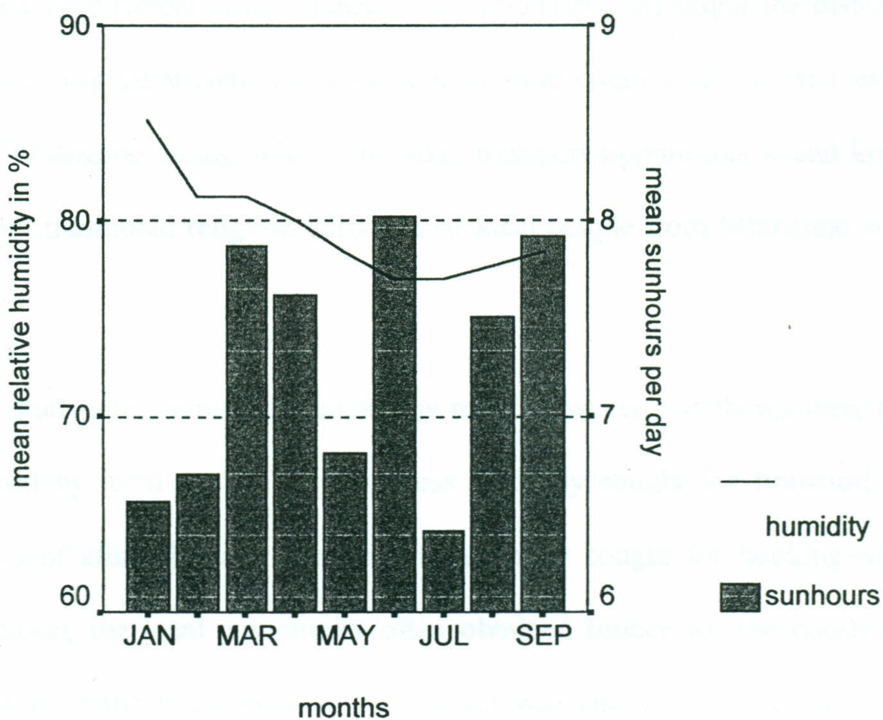


Figure 2.2 Mean humidity and mean sunshine during the study period.

Islands, Mzenzi Island, Risi Island and Mbasa Islands. Also, along the lakeshore of the mainland, an isolated thicket strip known as the Kisui-thicket was surveyed to become a control site (Table 2.1).

Being too large, Mfangano Island, Takawiri Island, Ngodhe Island, Saga Island, Mogari Islands, Kimaboni Island and Rusinga Island were considered to have a higher migration rate of tsetse flies then required. Sukuru and Kiringiti Island were known to be densely inhabited by man and completely deforested and no survey was therefore carried out.

Following the survey, the northern and southern Chamaunga Island, Wahondo Island and the Kisui-thicket were chosen for field experiments (Fig. 2.3). Thereby, different circumstances were considered including the distance from MPFS, rugged waves beginning around 1300 hours until the early evening at the Mfangano facing side of the lake, transport opportunities, and knowledge about traditional religious activities of local people from Mfangano at Mzenzi Island.

All study sites were not occupied by man. However, the shores were regularly fished by local people, more or less regularly sought for firewood, and the roots of alluvial Water hyacinth occasionally sought for hooking-worms. In addition, the local community often obtained timber for the construction of huts from the Kisui thicket. The thicket was also regularly used for grazing goats and cattle.

Table 1.1 Results of the initial survey for suitable study sites.

site	no. of trap days	flies / trap and day	sex-ratio
northern Chamaunga Island	3	18.3	1.39
southern Chamaunga Island	5	2	0.43
Sentinel Island	3	0	-
Wahondo Island	4	31.25	0.39
America Island	3	0.3	0
Northern Bridge Island	2	0	-
Southern Bridge Island	2	0	-
Mzenzi Island	6	87	0.69
Risi Island	4	0.25	1
northern Mbasa Island	5	0.2	0
southern Mbasa Island	5	2.6	1.6
Kisui-thicket	10	10.2	1.49

The favoured host of *Glossina fuscipes fuscipes* in the study area is reportedly the monitor lizard (*Varanus niloticus niloticus*). The lizard occurred in high numbers at all study sites. It was shown that up to 98 % of the identified blood meals were of the monitor lizard irrespective of cattle and human prevalence and season (Mohamed-Ahmed and Odulaja, 1997).

2.3.2 Chamaunga Islands

Chamaunga Islands are about 2.4 km east of Mbita Point and include two islands. The southern island is about 200 m and the northern one about 850 m off the mainland (Plate 2.1). The distance between both is about 450 m.

During the study period, the northern island was about 300 m long and 140 m wide, with a circumference of about 770 m. A small peninsula-like lowland part on the northern side was completely flooded due to heavy rains in the end of 1997. Along the length, the western side had a steep slope to the inner upland, while the eastern side had a lowland strip of about 15 m in width which rose slightly to the inner upland. The island rose slightly from north to south resulting in a steep drop on the southern slope. The island was almost completely covered with dense thicket vegetation. Some big fig trees grew along the shore, and some big *Euphorbia* plants inside the island. Extending grassland of about 150 x 40 m covered the centre of the island.

The southern island was almost circle-like and had a circumference of about 500 m and a diameter of 150 m. Along the shore, the island had a lowland

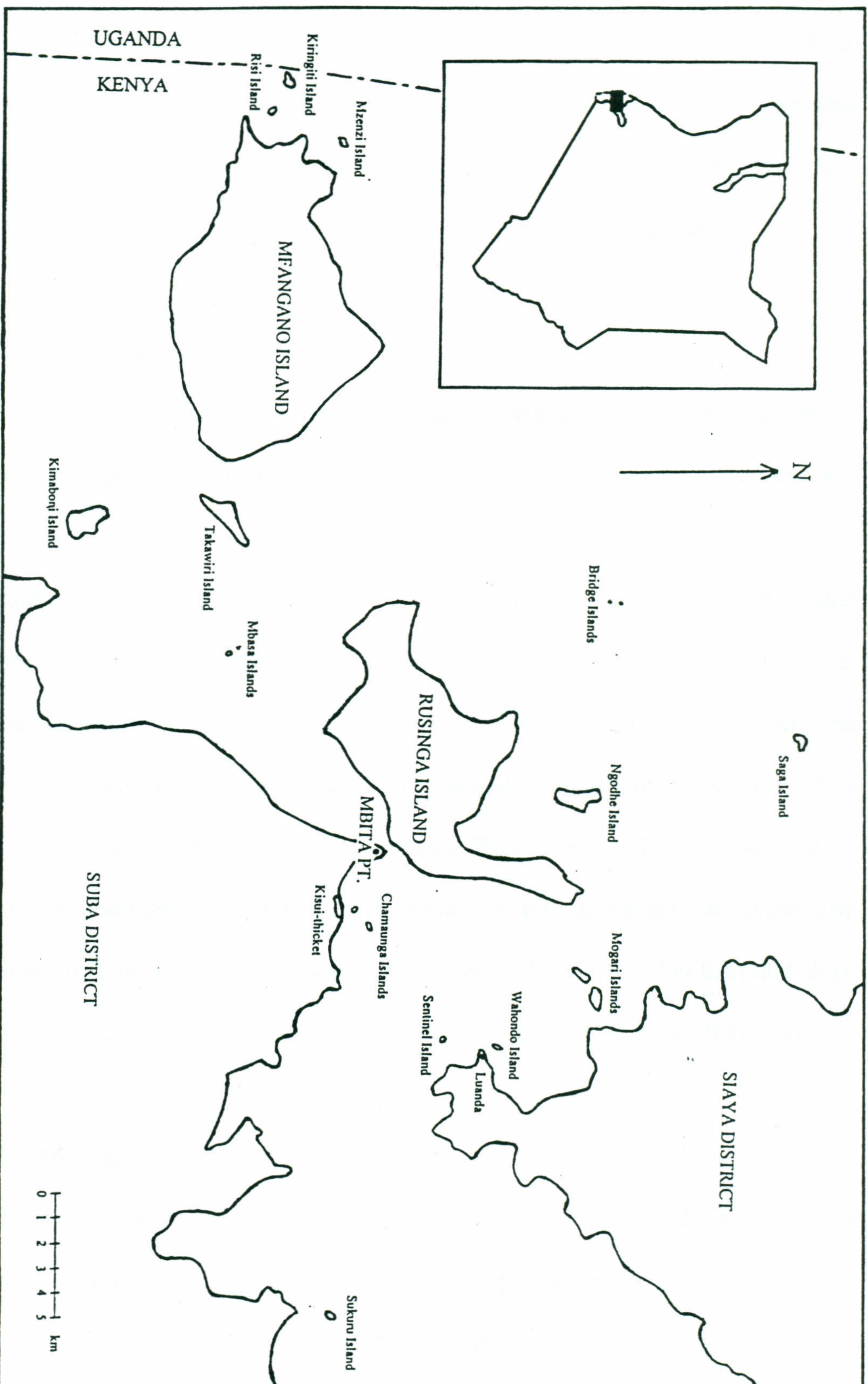


Figure 2.3 Map of the study area.

strip of about 20 – 40 m in width which moderately rises to an inner upland. About one third of the island was cultivated with maize and sorghum. Another third was grassland with some thicket vegetation. The rest of the island was covered with dense thicket, which was interrupted by grass portions.

2.3.3 Wahondo Island

Wahondo Island was about 8.5 north-east of Mbita Point and 800 m off the mainland near to Luanda / Siaya District. The island was about 230 m long and 100 m wide, with a circumference of about 600 m. Along the length on the southern and western side, the shore were covered with some low thicket vegetation and small single trees while rising steeply to the upland which was characterised by grassland and a few *Euphorbia* plants. The eastern and western side of the island consisted of lowland-like parts of about 40 x 100 m each which rose moderately to the upland. The eastern lowland and slope were all cultivated and planted with maize while the shore had a narrow thicket strip of about 5 m containing some single fig trees. The western lowland and slope were characterised by big fig trees and dense thicket vegetation (Plate 2.2).

2.3.4 Kisui-thicket

The thicket was along the lakeshore between Gombe and Kisui around 2.5 km east of Mbita Point. The dense thicket was approximately circa 1 km long and up to 150 m wide strip (Plate 2.1). It was interrupted irregularly by small

Plate 2.1 Bird's-eye view of the Chamaunga Islands and a part of the Kisui thicket from the south.

Plate 2.2 Wahondo Island showing the western lowland part which is characterised by big fig-trees with dense thicket vegetation

cultivated fields and grassland. Banana, lemon, fig and some other trees occurred in groups or singly. Some small non-permanent streams coming from Gembe Hills crossed the thicket into the lake.

2.4 References cited

- Kenya Island Mission, Inc. (KIM) (1997). *Prospectus*.
- Mohamed-Ahmed, M.M.; Odulaja, A, (1997). Diel activity patterns and host preferences of *Glossina fuscipes fuscipes* (Diptera: Glossinidae) along the shores of Lake Victoria, Kenya. *Bulletin of Entomological Research* 87: 179-186
- Mwangelwa, M.I.; Dransfield, R.D.; Otieno, L.H.; Mbata, K.J. (1990). Distribution and diel activity patterns of *Glossina fuscipes fuscipes* Newstead on Rusinga Island and Mainland in Mbita Point, Kenya. *Insect Science and its Application* 11(3): 315-321

CHAPTER THREE

EXPERIMENTS WITH FLUORESCENT PIGMENT POWDER DETECTING CONTACT EVENTS BETWEEN TSETSE FLIES

3.1 Introduction

The 'Dissemination Technique' is a method used to control insect pests. It is based on passing a sterilising or lethal agent from contaminated released insects to other target-insects through contact events (Masner *et al.*, 1968). This technique may be suitable for the control of tsetse flies. It has been shown that males can recognise the sex through contact pheromones in the wax layer of the female cuticle surface (Langley *et al.*, 1975; Carlson *et al.*, 1978). Furthermore, the only initial attraction seems to be visual (Wall, 1989), and there is no evidence for a long-distant sex attractant (Dean *et al.*, 1969; Turner, 1971, Wall, 1989) and sound attraction (Langley, 1977). That means, males may have to contact other specimens in order to recognise a potential mate. Therefore, contact events may generally happen between tsetse flies. In order to determine the potential of the 'Dissemination Technique', the number of contact events and the dependence on sex, age and population density were investigated. Fluorescent pigment powder was used to detect contact events in the field. This powder can be transmitted through contact from one fly to another (Turner, 1980). The field experiments were carried out in Kenya on three small islands in Lake Victoria with *Glossina fuscipes fuscipes.*

3.2 Materials and Methods

3.2.1 General laboratory experiments

The laboratory experiments were carried out with *Glossina austeni* and *Glossina fuscipes fuscipes* from the rearing unit of ICIPE in Nairobi. They were fed 5 days a week on cattle blood through a membrane before and throughout the experiment. Further experiments were carried out with *Glossina fuscipes fuscipes* freshly captured around MPFS as well as reared in the insectary at MPFS. They were fed daily on live shaved goats (Nash *et al*, 1968) before and throughout the experiment (Mwangelwa, 1992). *Glossina austeni* was reared in round metal cages (diameter 400 mm, height 30 mm), 30-50 individuals per cage. *Glossina fuscipes fuscipes* was reared either in round metal cages, 30-50 individuals per cage, or in PVC cages (oval 180 x 70 x 50 mm or round with diameter 140 mm, height 50 mm), 15-20 individuals per cage.

3.2.2 Effect of marking

The objective of this study was to establish the effect of fluorescent pigment powder (Bio Quip catalogue '95) and acrylic colour (Fevicryl by Pidilite) used for the field experiments as well as the corresponding handling procedures on tsetse flies. The underlying assumption of this laboratory experiments was that these effects did not depend on the *Glossina* species or sex. Thus, pigment powder was sufficiently applied at and around the coxae. Acrylic colour was applied as a small dot on the dorsal side of the thorax. It was assumed that handling while applying the pigment powder would affect the flies more than

handling while applying the acrylic colour, and therefore, no handling control was set for the acrylic colour. All flies of each experiment were kept under the same environmental conditions in PVC cages used for rearing, 10 individuals per cage. The mortality was recorded daily until all flies died, but not longer than 30 days. The experiments were arranged as follows.

(a) 50 randomly selected wild females of *G. fuscipes fuscipes* captured 2-4 days earlier were each handled without marking ($_H$), marked with yellow ($_Y$) and red pigment powder ($_R$) and tested against a not handled control.

(b) 50 randomly selected 4-7 days old reared males of *G. austeni* were each marked with red ($_R$) and green acrylic colour ($_G$) and tested against a not handled control.

(c) 50 randomly selected wild females of *G. fuscipes fuscipes* captured 2-4 days earlier were each handled without marking ($_H$), marked with yellow pigment powder and yellow acrylic colour ($_Y$) and were tested against a not handled control.

3.2.3. Transmission tests with fluorescent pigment powder

The objective of the study was to investigate the transmission of the used fluorescent pigment powder from one fly to another in order to determine the proper duration of the field experiments. The underlying assumption of these laboratory experiments was that the quality of marks caused by transmission did not depend on the species of *Glossina* but only on the powder and the contact event. In case of *Glossina austeni,* 30-day-old males and 1-day-old virgin females were used. In case of *Glossina fuscipes fuscipes,* randomly

chosen males from 1-day-old wild catches, and reared 1 to 6-day-old virgin females were used. Any of the two species was used depending on which was easier available during the study period. Males were marked at and around the coxae and females dorsal on the meso- and metathorax. One hour after marking, one male and female were placed together in a PVC cage used for rearing. Thereby, only one of them was originally marked. Then, the cage was tapped slightly in order to induce a copulatory contact. Immediately after they got in the typical copulatory position, they were forced to separate by tapping the cage hardly (brief contact). Afterwards, the flies were kept in small PVC boxes which had a net cover (height 35 - 40 mm, diameter 26 - 31 mm). The originally unmarked flies were daily examined for fluorescent marks using a UV light (Versalume PP-FLS Long-wave/Short-wave by Biotech), while holding them with two fingers. A sucking tube was used to pick the flies. Thereby, after handling one fly the used sucking tube was cleaned with a piece of textile. Fluorescent pigment powder was detected in complete darkness. Five marking categories have been distinguished: 1. 'unmarked' 2. 'very poorly marked' (1-3 single pigments) 3. 'poorly marked' (4-15 single pigments) 4. 'well marked' (more than 15 single pigments up to a spot of pigments covering a size of the half scutellum) 5. 'very well marked' (pigment spots covering more than a size of the half scutellum). The experiments were arranged as follows.

(a) The marking effectiveness of yellow, red and blue pigment powder was tested. For each colour, 20 marked males were briefly contacted with each

one unmarked female. The females were examined up to day three after contact.

(b) 20 yellow marked males were briefly contacted for 4 subsequent days with one unmarked female. The females were examined up to the 3rd day after contact.

(c) 5 yellow marked males were briefly contacted with each 10 unmarked females within 2-3 hours. The females were examined up to day 3 after contact.

(d) 30 yellow marked females were briefly contacted for 4 subsequent days with one unmarked male. The males were examined up to day three after contact.

3.2.4 Determining the capture procedure for the field experiments

This study was aimed at determining whether the flies have to be captured individually during the field experiments in order to ensure that there is no artificial transmission of the fluorescent pigment powder through contact in the cage. Therefore, the following experiment was carried out with *G. fuscipes fuscipes*. Four males captured two days earlier were marked at and around the coxae with fluorescent pigment powder and on the thorax with acrylic colour. They were then kept individually in small PVC boxes. After 24 hours, each was transferred to a cage, which is usually mounted on a pyramidal trap. To each cage 30 wild unmarked males captured 2 – 4 days earlier were added. Observations were made as to whether the marked males attempted to mate the unmarked males. After one hour, all flies were killed using ether. The

originally unmarked males were then examined for marks of fluorescent pigment powder.

3.2.5 Arrangement of the field experiments

The objective of the field experiments was to investigate the number of contact events and the dependence on sex, age and population density. The experiments were carried out during sunny weather. The experimental sites were on the eastern lowland strip of the northern Chamaunga Island, which was completely covered by dense thicket vegetation and one big fig-tree on the edge of the site, on the eastern side of the southern Chamaunga Island, which was covered by dense thicket vegetation, and on the western low-land part of Wahondo Island, which was characterised by some big fig-trees and dense thicket vegetation. During the first field experiment, males were marked and released (exp. I), which was repeated three times (exp. II, III, IV) at different population densities. In another experiment, females were marked and released (exp. V). The experiments were arranged as follows.

I. Carried out in March '98 on Wahondo Island with yellow pigment powder at medium-level population density.
II. Carried out in April '98 on the northern Chamaunga Island with red pigment powder at high-level population density.
III. Carried out in May '98 on the southern Chamaunga Island with yellow pigment powder at low-level population density.
IV. Carried out in May '98 on the northern Chamaunga Island with yellow pigment powder at high-level population density.

V. Carried out in May '98 on the northern Chamaunga Island with red pigment powder at high-level population density.

3.2.6 Marking and releasing of flies

At each experimental site, a sufficient number of flies were captured using pyramidal traps. The cages were emptied every 1-2 hours. Thereafter, they were fed still in the field on a live shaved goat. Then, 125 males or females respectively were marked with fluorescent pigment powder for transmission purposes and with acrylic colours for easy identification while on the island. Fluorescent pigment powder was applied at and around the coxae of the males and dorsal on the meso- and metathorax of the females. Acrylic colour was applied as a small dot on the dorsal thorax. After marking, they were kept individually in small PVC boxes to avoid any contact before releasing. About 30 minutes after sunset, the marked flies were released in the middle of the experimental site. In case of the ultra low density experiment of the southern Chamaunga Island, 62 fed flies were marked in the laboratory. They were captured one day earlier on the northern Chamaunga Island. They were then released about 30 minutes after sunset.

3.2.7 Capture and treatment of flies

On the second day after release, flies were captured from 0630 hours to 1800 hours with 5 pyramidal traps. The traps were placed along the shoreline at a distance of about 30 m between each other, with trap number three (the medium trap) set exactly at the release point. Experiment IV was carried out

with three traps due to logistical problems. Each trap was served by one assistant, who was hidden about 10 m from the trap. With each captured fly, the cage was immediately replaced with a new one in order to capture all flies individually. That was to avoid any artificial transmission of the fluorescent pigment powder through contact between the flies in the cage and to ensure that no powder, which may have been lost in the cage by one fly, was picked up by a following fly. The flies were killed in the cage. Originally unmarked flies were killed by squeezing the prothorax with tweezers and placed head first in a small closeable PVC vial. The PVC vials were labelled with the number of the trap and of the fly. Additionally, when flies were accidentally captured together, their individual number was noted. Originally marked flies were put away.

3.2.8 Determination and evaluation of contact events

All captured flies, which were originally unmarked, were examined in the laboratory on the same day for marks of transmitted fluorescent pigment powder. Therefore, a 6th marking category 'medium marked' (8-15 single pigments) was introduced and the category 'poorly marked' reduced to 4-7 single pigments. The marking category and marked body-parts were noted for each fly in order to determine the quality of the marks and the kind of transmission. Flies were noted according to their marks either as 'have contacted', 'were contacted' or both which was based on the normal copulatory position with the active mating partner being on top of the passive one. The terms 'F0-, 'F1- and 'F2-generation' used in this study apply on horizontal

transmission but not vertical transmission with 'F0-generation' being the originally marked flies.

A fly was noted as 'has contacted':

- if the only mark was ventral.
- if it was ventrally 'very well marked' or 'well marked' and dorsally 'poorly marked'.

A fly was noted as 'has contacted' and 'was contacted':

- if it was ventrally and dorsally marked in the same quality, but no fight was assumed to have taken place (no lateral mark).
- if one side was 'very well marked, 'well marked' or 'poorly marked' and the other side 'medium marked', but no fight was assumed to have taken place (no lateral mark).

A fly was noted as 'was contacted':

- in all other imaginable mark situations such as dorsally and laterally marked.

Flies which were marked through transmission were excluded from the analysis in following cases:

- category 'very poorly marked' was assumed to be an artefact through handling or artificial transmission in the trap itself.

- when two flies were accidentally captured together and one of them was originally marked, since a contact event may have taken place in the cage.
- when two originally unmarked flies were accidentally captured together and one of them was 'very well marked' or 'well marked' and the other one only 'medium marked' or 'poorly marked', since a contact event may have taken place in the cage.
- when the fly was only marked on the prothorax, since it was squeezed using tweezers, which might have artificially transmitted powder from one fly to another.

There was a special case of evaluating the marking characteristics. That was when males, which were marked through transmission, were totally covered with fluorescent pigment powder. Therefore, it was assumed they were involved in a fight. Since males, which were marked all over through transmission were only noted in category 'well marked' and 'very well marked', it was additionally considered that only a fight between males of the 'F0-' and the 'F1-generation' but not between 'F1-' and the 'F2-generation' could have taken place to cause such a good marking quality. In this case, there were theoretically two possibilities to get such a mark. That is, either the originally marked male approached the originally unmarked male or vice versa. Since every male had the same chance to contact as well as to get contacted, the number of so marked males was simply halved and one half considered as 'has contacted' the other half as 'was contacted'.

In case of experiment I, only the dorsal and lateral parts but not the ventral parts of the flies were examined. Therefore, only flies which 'were contacted' were considered. For that reason, theoretically there may have been flies in this experiment, which were dorsally 'poorly marked' but would have been noted in the other experiments as only 'have contacted', because of the good marking quality on the ventral side. Yet, in the other experiments only 2 specimens were noted as only 'have contacted', although they were dorsally 'poorly marked'. Therefore, it was considered that this category can be neglected for experiment I.

3.2.9 Determination of age

The flies' age was determined according to the wing-fray method of Jackson (1946) using a dissecting microscope. The wing-fray category was noted according to the individual number of the fly. It was also noted whether flies were tenerals or not. A teneral was considered as such, if the ventral side appeared whitish and semi-transparent, the body had a soft feel and the ptilinum could be everted when the sides of the head were squeezed between the fingers.

3.2.10 Preferences of contacting males

Initial analysis have shown that there was no population density dependence of preferences for certain wing-fray categories of males and females respectively. Therefore, the data were pooled in order to increase the number of flies for statistical analysis. The number of males and females respectively of each

wing-fray category, which 'were contacted' and which 'were not contacted', was compared with the number of each other wing-fray category. Furthermore, the pooled number of males of each wing-fray category, which 'were contacted' and which 'were not contacted', were compared with the corresponding number of females of each wing-fray category.

3.2.11 Sexual activity of males

Initial analysis had shown that there was no population density dependence of the sexual activity of males. Therefore, the data were pooled in order to increase the number of flies for statistical analysis. The number of males of each wing-fray category, which 'have contacted' and which 'have not contacted', was compared with the number of each other wing-fray category of the same experiment.

3.2.12 Calculating the average contact rate per male and day

In order to calculate the average contact rate per male and day for experiments I – IV, the following situation was assumed:

- The catchment basin around the traps represented a closed population (no emigration and immigration rate).
- The capture probability of all flies was identical.
- The mortality rate of all flies was identical.
- Every contact of the originally marked flies caused a transmission of the pigment powder.

- Transmitted powder remained on the fly up to the end of the experiment.
- No double contacts occurred (a fly could get marked through transmission only once).
- No contact events occurred on the day of recapture.
- The number of flies which 'were contacted' derived only from horizontal transmission from the 'F0-' to the 'F1-generation'.

Therefore, the average contact rate per male and day can be calculated by dividing the total number of contacted flies with the number of recaptured originally marked males. The chance for an originally marked male to get contacted was the same as for an originally unmarked male. However, there was no way to detect whether an originally marked male was contacted. Therefore, the percentage of contacted originally unmarked males was used to calculate the number of contacted recaptured originally marked males. The so calculated number of contacted originally marked males was added to the number of contacted originally unmarked flies resulting in the total number of contacted flies.

The average contact rate per male and day was calculated as follows:

$$\frac{(a+b) + \frac{a\ c}{d}}{c}$$

a = number of contacted originally unmarked males
b = number of contacted originally unmarked females
c = number of recaptured originally marked males
d = number of all originally unmarked males

In order to compare the calculated average contact rates per male and day, the following situation was assumed:

- During all field experiments the same biotic and abiotic factors were prevalent.
- Males contacted other tsetse flies regardless of sex and age.

3.2.13 Statistical analysis

The laboratory tests for the effect of pigment powder as well as the combination of pigment powder and acrylic colour and the corresponding handling procedures were analysed using the Mann-Withney U-test with Monte Carlo significance. The laboratory tests of the effect of acrylic colour on survival were analysed using the Kaplan-Meyer procedure. The wing-fray categories and the sexes were compared regarding the portion of unmarked flies and the portion of flies which were marked through transmission using the chi square test. All data were analysed using SPSS 6.12 software.

3.3 Results

3.3.1 The effect of marking

The pigment powder and the corresponding handling procedure had no significant effect (P_Y= 0.283, P_R= 0.217, P_H= 0.304) on the tsetse flies (Fig. 3.1). Similarly, the acrylic colour had no significant effect (P_R= 0.865, P_G= 0.424) (Fig. 3.2a). The combination of the pigment powder and acrylic colour as well as the corresponding handling procedure had also no significant effect on the tsetse flies (P_Y= 0.635, P_H= 0.201) (Fig. 3.2b).

3.3.2 Transmission tests with fluorescent pigment powder

For a) all females were marked up to day three after contact using the three colours of fluorescent pigment powder. The quality of the yellow and red marks was better than the blue marks (Tables 3.1 – 3.3). For b) all females were marked up to day three after initially marking the males (Table 3.4). For c) all females were marked up to day two after contact. On day three after contact only 92 % of the females were still marked (Table 3.5). For d) all males were marked up to day three after initially marking the females (Table 3.6). The number of tested females dropped drastically on the 1st and 2nd day after initially marking the females, because males refused to mate with them. The originally unmarked females were dorsally marked on the thorax and the wings and occasionally dorsal on the abdomen and lateral on the thorax. The originally unmarked males were marked at and around the coxae and femur and occasionally on the tibia, tarsae and ventral on the abdomen. Generally, the

quality of the transmitted marks declined with days after marking the 'F0-generation', with days after contact and with number of contacts.

3.3.3 Determining the capture procedure for the field experiments

No mating attempt of the originally marked flies was observed. However, an average 44.2 % of the originally unmarked males were noted in category 'very poorly marked', 39.2 % in category 'poorly marked', 4.2 % in category 'well marked' and non in category 'very well marked' totalling to an average of 87.5 % males which were marked through transmission.

3.3.4 Overall results of the field experiments

The overall results from all 5 field experiments are presented in Table 3.7. The recapture rates were between 11.3 to 25.6 %. The detected sex ratio was in all experiments below one, suggesting a higher proportion of males. In all field experiments, contact events were detected except at ultra low-level population density (exp. III). Females which 'have contacted' were only noted twice, though only as 'poorly marked'. Therefore, the results show that females generally exhibit passive sexual behaviour. With only males exhibiting active sexual behaviour, transmissions of pigment powder were detected from the 'F0-' to the 'F1-generation' as well as from the 'F1-' to the 'F2-generation'. However, when males were originally marked, the percentage of contacted flies was higher (20.7 to 24.1 % for females; 15.3 to 23.7 % for males) than in the case of originally marked females (10.0 % for females; 6.4 % for males). This shows a better transmission from the 'F0-' to the 'F1-generation' than

from the 'F1-' to the 'F2-generation'. Similarly, if females were originally marked, the percentage of contacting males was higher (22.9 %) than in case of originally marked males (3.9 and 9.5 %).

3.3.5 Preferences of contacting males

All captured tenerals were noted in wing-fray category 1. Therefore, wing-fray category 1 was distinguished in tenerals (1T) and non-tenerals (1NT). There was no significant difference ($P < 0.05$) between the wing-fray categories of the contacted females (Table 3.8). In case of contacted males, there were significant differences ($P < 0.05$) between tenerals and non-tenerals of wing-fray category 1 and wing-fray category 2 (Table 3.8). The differences appeared to be caused by discrimination against tenerals and non-tenerals of wing-fray category 1 (Fig. 3.3). When pooled data of males were compared with pooled data of females, significant differences ($P < 0.05$) were detected between female and male tenerals, between female tenerals and male non-tenerals of wing-fray category 1, between females of the wing-fray category 4 and male tenerals as well as between females of the wing-fray category 4 and male non-tenerals of wing-fray category 1 (Table 3.9). The differences appeared to be caused by preference for female tenerals and females of wing-fray category 4 and by discrimination against male tenerals and non-tenerals of wing-fray category 1. (Fig. 3.3).

3.3.6 Sexual activity of males

Significant differences ($P < 0.05$) were detected between tenerals and non-tenerals of wing-fray-category 1, 2, 3, 4, 5, between non-tenerals of wing-fray-category 1 and wing-fray-category 2, 3, 4, 5, between wing-fray-category 2 and 3 and between wing-fray-category 5 and 6 (Table 3.10). No teneral and no male of wing-fray category 6 was found to have actively contacted (Fig. 3.4).

3.3.7 Average contact rates per male and day

For experiment I to IV the average contact rates per male and day were calculated as follows: (I) 0.75 contacts (18 + 6 / 32), (II) 1.94 contacts (57 + 5 / 32), (III) no contacts (0 / 7) and (IV) 2.81 contacts (54 + 5 / 21). The calculated average contact rate per male and day was strongly positively correlated with population density ($P < 0.01$, $r = 0.992$) (Fig. 3.5).

3.4 Discussion

3.4.1 Laboratory experiments

The used pigment powder and acrylic colour as well as the handling procedure were shown to be harmless to the flies. The fluorescent pigment powder used was transmitted through contact from males to females, which generally confirmed Turner's (1980) results. In the present study it was also shown that transmissions during a brief contact for only a few seconds were successful. Furthermore, the powder was transmitted successfully, when the male contacted several females within a few hours. Red and yellow pigment powder marked better than blue pigment powder. In addition, the transmission was proved successful even from females to males after brief contact. Thereby, the powder stuck after 24 hours mainly in the groove between the dorsal meta- and postthorax of the originally marked females. However, the results showed that field experiments using fluorescent pigment powder, which was to detect contact events, should last as short as possible in order to estimate a daily male contact rate. This is because the quality of marks decreased with time after initial marking the 'F0-generation', the time after contact and the number of contact events. That was why, the field experiments were designed such that one day after the first possible contacts the flies were collected and examined. Furthermore, it was shown that it is absolutely necessary to capture the flies individually when using fluorescent pigment powder.

3.4.2 Preferences of contacting males

In this study, it was observed that male tsetse flies generally contacted other tsetse flies regardless of sex and age except in some few cases suggesting that the morphological differences between males and females are too small to be detected by the males. It suggested further that mate seeking males are attracted preliminary visually and that they may finally identify the sex and the willingness via contact. However, the results do not lead necessarily to the proposed contact pheromones (Langley *et al.*, 1975; Carlson *et al.*, 1978; Huyton *et al.*, 1980 a, b). That is because, contacting males could theoretically distinguish only between a potential mate (willing female) and the rest (non-willing female or male) simply through the receptive or refractory behaviour of the target fly. Thereafter, the copulatory response of the male could be caused only by the willingness of the female.

The observed male/male contacts could have been a result of mate seeking as well as aggressive behaviour. However, cases where the marking characteristics of contacted males suggested a male/male fight were very rare, approximately 9.0 %. Furthermore, both copulatory and simple aggressive intentions could have triggerred such a fight. Therefore, male/male contacts were considered to be a result of sexual behaviour.

Vanderplank (1947) established in the laboratory cross mating between *G. morsitans* and *G. swynnertoni*. The results of the present study suggested

further that there may be regular contact in the field between specimens of different tsetse species of about the same size and occurring at the same site.

Although, male tsetse flies generally contact other tsetse flies regardless of sex and age, there are a few exceptions. There seemed to be a preference for female tenerals, which were the youngest females, and females of wing-fray category 4, which were the oldest found in this study. In addition, there seemed to be a discrimination against male tenerals and non-tenerals of wing-fray category 1, which were the youngest males. Theoretically, there are several explanations for these preferences or discriminations including appearance, behaviour and volatile chemicals of the flies.

The visible special characteristics of tenerals (the ventral side appears whitish and semi-transparent) appear to be too slight. Therefore, it seems unlikely that males can visually identify those newly emerged flies. Otherwise, the appearance of non-tenerals does not change during a flies life. Therefore, appearance could not have caused the preferences and discriminations.

If volatile chemicals are considered to cause the preferences and discriminations, then they could have been self-produced or residuals of the puparium as in case of the youngest flies. In case of female tenerals, both explanations may be possible. In case of young males there seemed to be no advantage when they actively produce repellent chemicals. Therefore, a residual of the puparium may be more likely. This was supported by the

evidence that teneral males were even more discriminated than non-tenerals of wing-fray category 1 (Fig. 12) indicating that the residual of the puparium may loose its efficiency with time. In case of females of wing-fray category 4 the volatile chemicals could be only self-produced. Olfactory chemicals would be of great importance. For example, a volatile attractant could lead to higher attraction to devices like traps or targets. However, more important could be an effective volatile repellent, which would prevent tsetse flies from feeding on cattle or humans and therefore from transmitting the trypanosomes.

On the other hand, the preferences and discriminations could be simply a result of behaviour including sound attraction and increased or decreased presence at sites, where males aggregate. The youngest females may naturally seek for the first mate, while the oldest females may need to seek a mating partner in order to restore the spermatheca due to loss of sperm fitness. Mate seeking may be necessary because male tsetse flies are able to recognise a moving or stationary objects of a size of a female only within 20-25 cm (Brady stated by Wall, 1989). This courtship behaviour may generally occur around host animals (Langley and Hall, 1984). This was concluded because male aggregates, known as the 'following swarm', were severally observed around moving objects (Swynnerton, 1936; Bursell, 1961; Brady, 1972; Vale, 1974) and it was shown that females which are not hungry occur around baits (Vale, 1974). All males of these aggregates may be potential mates for the mate seeking female, because males mate at any stage of the hunger cycle (Langley and Hall, 1984). Mate seeking around hosts may have caused the detected

preference for the oldest females. However, female tenerals of *G. fuscipes fuscipes* are mostly already inseminated when feeding for the first time (Saunders, 1962; Harley, 1966), which makes mate seeking around host animals unlikely for their preference. Yet, the preference for the youngest females may be caused by an aggregation of males around breeding sites (Challier, 1982). The discrimination against the youngest males may be because they may avoid male aggregations for one reason or another. Theoretically, sound production could also attract and repel a potential mate. However, there is no evidence for attraction through sound production in tsetse flies (Langley, 1977). Furthermore, there is no report known to me revealing repellent sound production, and it seems unlikely that only young males would do so.

In conclusion, the reason of the preference for the youngest and the oldest females and the discrimination against the youngest males is not clear being most probably either volatile chemicals or the behaviour of the flies. Yet, there may exist an olfactory repellent and attractant, which deserves further investigations.

The observed preference for females of wing-fray category 4 in the present study suggested multiple mating since those old females were certainly already inseminated. Furthermore, some cases of the better marking qualities may have resulted from real mating events even in the lower wing-fray categories. Therefore, non-teneral females were considered to have been non-virgins,

because tenerals were reported to be inseminated with a rate close to 100 % when captured for the first time (Saunders, 1962; Harley, 1966). However, with the present experimental design, it could not have been directly detected whether non virgin females which were marked through transmission were just briefly contacted or tried to mate or really mated. As proposed by Turner (1980), direct experiments would have to involve investigations about the 'topping-up' of the spermathecae through additional sperm transfers. Multiple mating may be advantageous when competition and selection between individual spermatozoa occur during egg fertilisation (Jaenson, 1980). Multiple mating has been demonstrated in the laboratory (Vanderplank, 1947; Dame & Ford, 1968; Jaenson, 1979b), and there is a few evidence of multiple mating in the field (Vale, 1974; Jaenson, 1980; Rogers, 1973b).

3.4.3 Sexual activity of males

Male tenerals and the oldest males in wing-fray category 6 seemed to be sexually inactive. The youngest non-tenerals in wing-fray category 1 seemed sexually active, though, not much. Males in wing-fray categories 2 - 5 seemed to be most sexually active. The inactivity of the very young males agrees with field observations done with artificial decoys placed on screens (Hall and Langley, 1989). Moreover, laboratory studies have shown that the activity and aggressiveness of males in terms of mating behaviour increase with age in the first 1-2 weeks (Nash, 1955; Jaenson 1979a). The increasing sexual activity is related to the development of the male accessory reproductive gland (Davies-Cole, 1990). In case of the oldest males, activity may suddenly decline. On the

other hand, Rogers (1973a) found that some males are more sexually active than others. If this higher persistence and aggressiveness would cause more wing-fray, then wing-fray categories 3, 4 and 5 would contain the more aggressive and active part of the male population. In case of wing-fray category 6 these aggressive males may have already died due to their energy consuming behaviour or the activity simply declines. Both explanations may overlap.

3.4.4 Average contact rate per male and day

The average contact rate per male was shown to be quite low with a few contacts per day. However, it might be higher, because some very glancing physical contacts may have only involved the tarsi and tibia of the contacting male where the chemoreceptors for the contact pheromone are assumed to be located (Langley *et al.*, 1987). Therefore, no powder may have been transmitted from the coxae of the male. Furthermore, some of these cases may have occurred, which were excluded from analyses but which would have increased the number of calculated contacts. For instance, some marks of lower quality may have been lost after one day and a few double contacts may have occurred. Furthermore, some flies of category 'very poorly marked' may have originated from natural contacts but not from artificial contamination. Thereby, the number of contacted flies in category 'very poorly marked' was only approximately half of the number of category 'poorly marked' to 'very well marked'. On the other hand, contacts certainly occurred also on the recapture day, which lead to an overestimation of daily contact events. In any case, the

natural male contact rate seems not to exceed a few encounters per day. In comparison, Wall (1989) counted only 15 contacts per male in 8 hours when exposed to 16 decoy females in a cage as small as 31 x 27 x 25 cm.

The number of contact events may be density dependent because more flies simply increase the probability for a mate seeking male to see another tsetse fly. Furthermore, Langley and Hall (1984) has shown that the duration of copulatory response of males to pheromone-baited decoys declined with increasing population density. Therefore, at higher population density, mating males may feel somehow disturbed, terminate mating and may later try to mate with another female.

3.4.5 Potential of the 'Dissemination Technique'

In conclusion, these results are very promising for the control of tsetse flies with the 'Dissemination Technique'. Although, the male contact rate was shown to be relatively low, one male or female could theoretically transmit an agent to several other flies within few days with the contact rate being positively linear correlated with the apparent population density. The technique of mounting a contamination device on a trap could increase the efficiency of a trap markedly and so reduce the number of necessary traps and hence the costs and time of serving them. However, since contact events are very rare at an ultra low population density, no eradication may be achieved using this technique alone. The success of releasing reared males might be limited, because the youngest males seem to contact other tsetse flies only in rare cases.

In case of coexisting tsetse species, only contaminated males of that species could be released, which is easier to rear provided that the males can not detect the difference of their sizes.

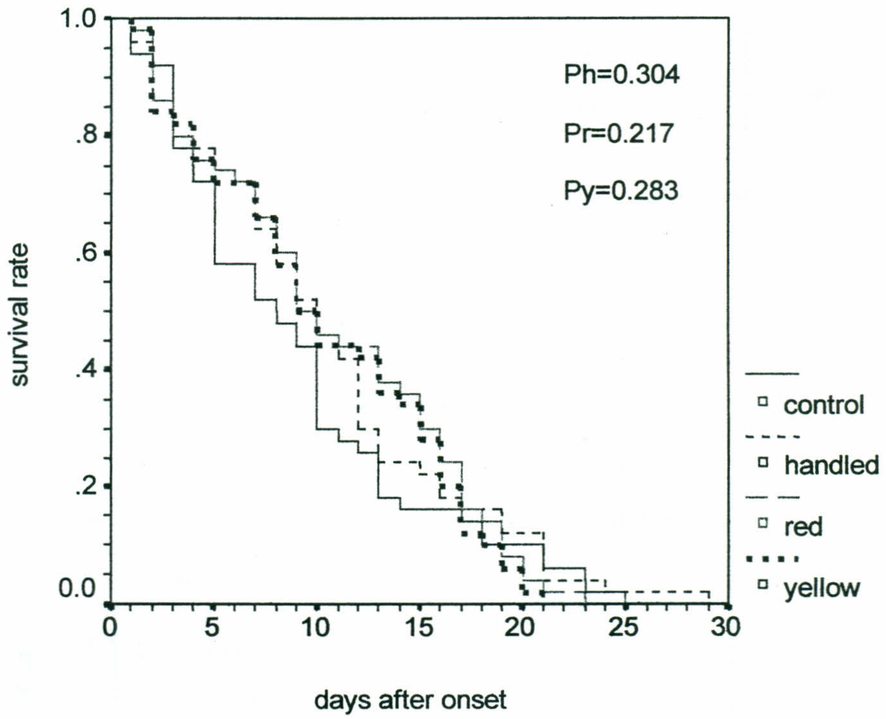
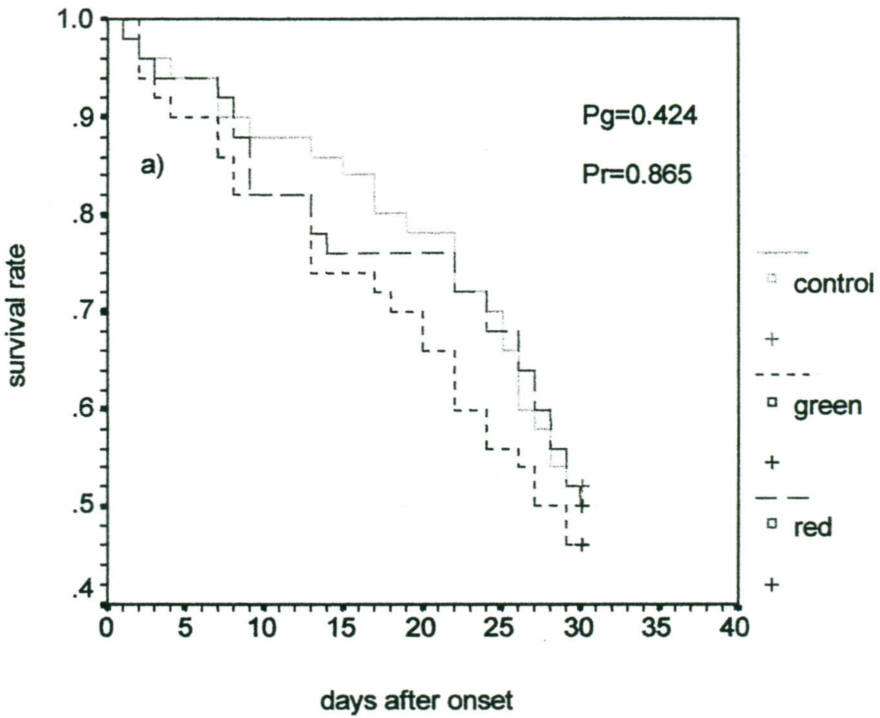


Figure 3.1 Survival rates of flies due to the effects of red and yellow fluorescent pigment powder and handling.



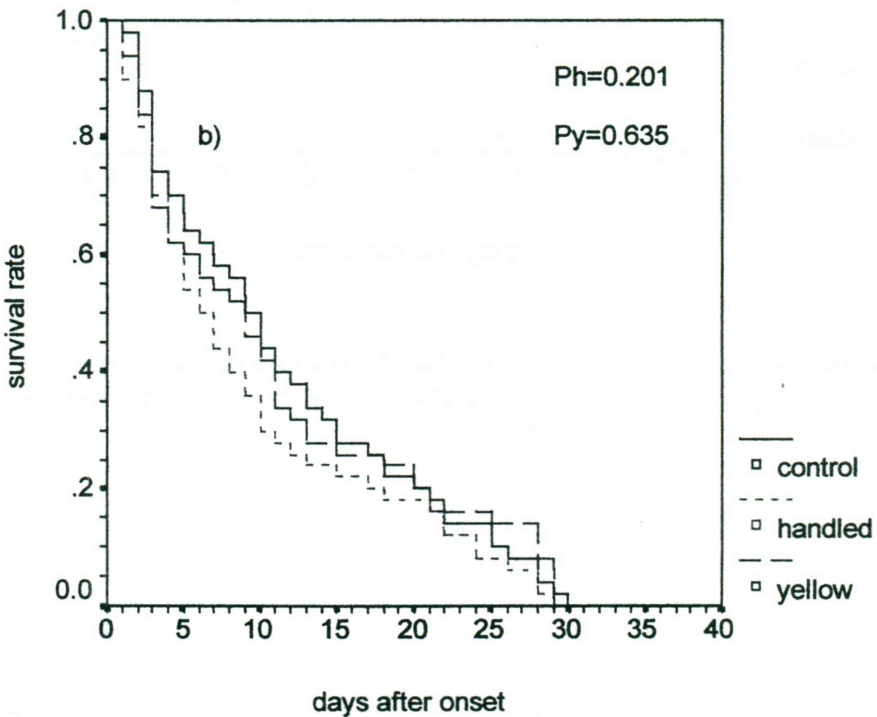


Figure 3.2 Survival rates of flies due to effects of a) green and red acrylic colour and b) of the combination of yellow pigment powder and yellow acrylic colour and handling.

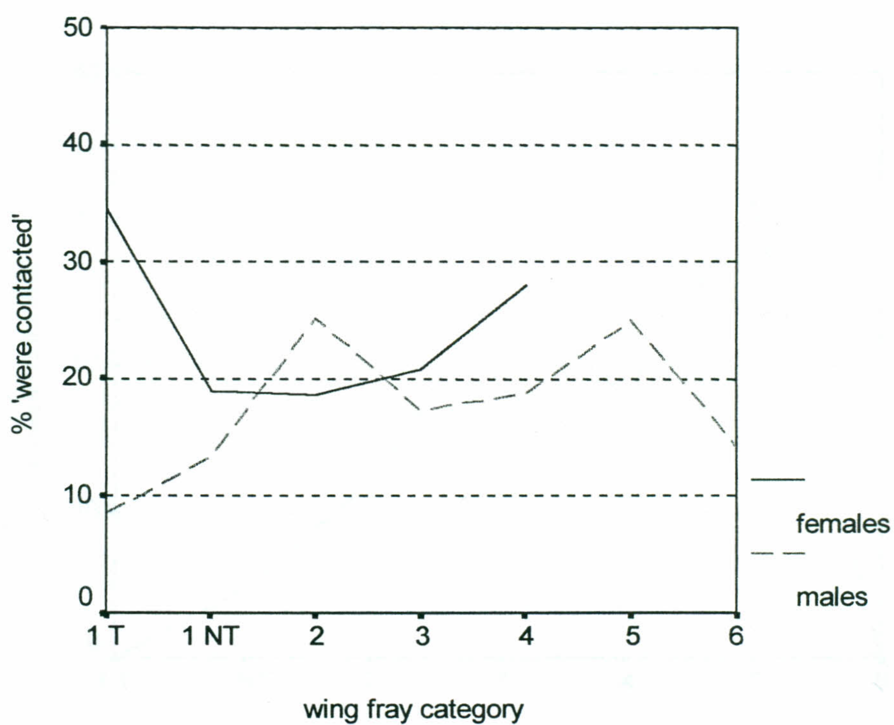


Figure 3.3 Percentage of males and females which 'were contacted' for each wing-fray category for experiments I, II, IV and V.

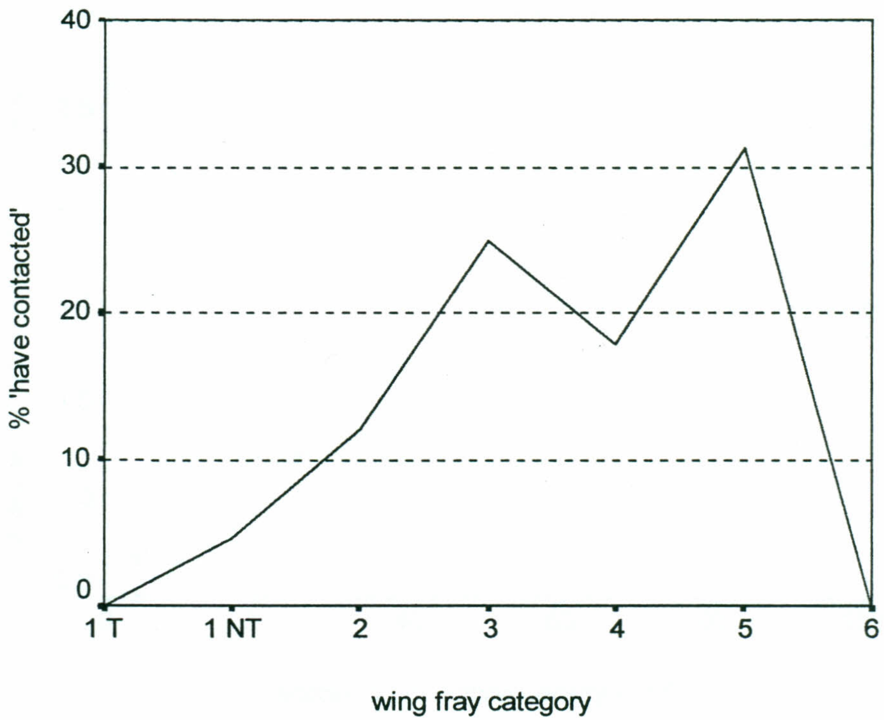


Figure 3.4 Percentage of males which 'have contacted' for each wing-fray category for experiments II, IV and V.

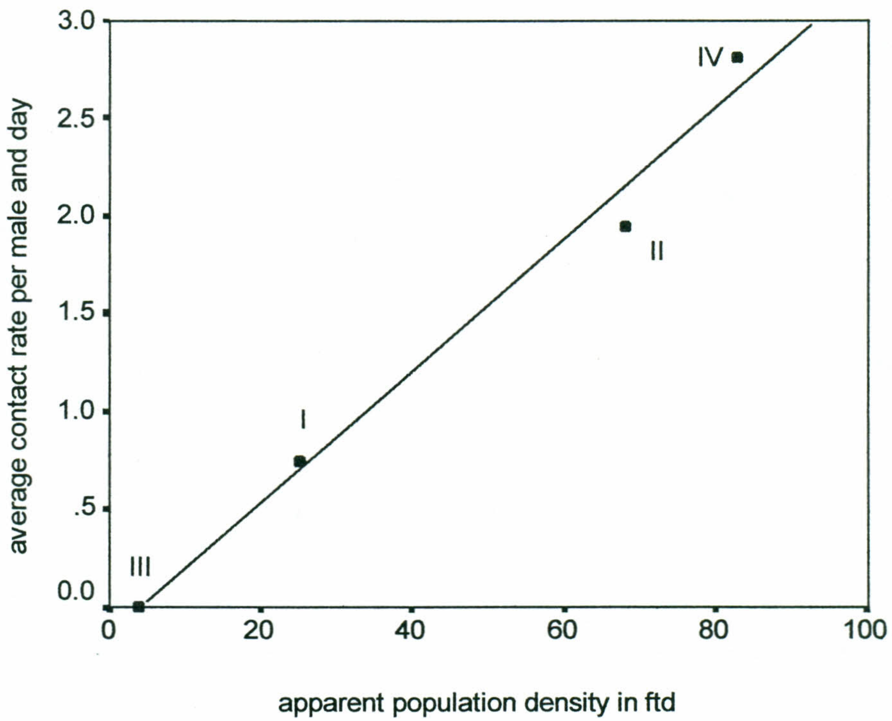


Figure 3.5 Correlation between the average contact rate per male and day and the apparent population density in ftd (flies per trap and day) for field experiments I - IV.

Table 3.1 Marking qualities on 4 subsequent days after male to female transmission of yellow fluorescent pigment powder by brief contact.

day after contact	no. of females examined	unmarked	very poorly marked	poorly marked	well marked	very well marked	% of females marked
-	20	-	-	-	8	12	100
1	20	-	-	-	8	12	100
2	19	-	-	2	7	10	100
3	17	-	-	3	5	9	100

Table 3.2 Marking qualities on 4 subsequent days after male to female transmission of red fluorescent pigment powder by brief contact.

day after contact	no. of females examined	unmarked	very poorly marked	poorly marked	well marked	very well marked	% of females marked
-	20	-	-	-	5	15	100
1	18	-	-	-	6	12	100
2	17	-	-	1	6	10	100
3	15	-	-	2	5	8	100

Table 3.3 Marking qualities on 4 subsequent days after male to female transmission of blue fluorescent pigment powder by brief contact.

day after contact	no. of females examined	unmarked	very poorly marked	poorly marked	well marked	very well marked	% of females marked
-	20	-	-	-	13	7	100
1	18	-	-	6	7	5	100
2	18	-	-	8	6	4	100
3	16	-	2	6	5	3	100

Table 3.4 Marking qualities of male to female transmission of yellow fluorescent pigment powder on 4 subsequent days by brief contact.

day after initially marking	no.of males mated	unmarked	very poorly marked	poorly marked	well marked	very well marked	% of females marked
-	20	-	-	-	9	11	100
1	20	-	1	3	5	11	100
2	18	-	3	4	6	5	100
3	17	-	-	4	6	7	100

Table 3.5 Marking qualities on 4 subsequent days after transmission of yellow fluorescent pigment powder from 1 male to 10 females within 2-3 hours by brief contact.

days after contact	no.of females examined	unmarked	very poorly marked	poorly marked	well marked	very well marked	% of females marked
-	50	-	-	7	15	28	100
1	50	-	5	18	20	7	100
2	45	-	5	18	19	3	100
3	42	3	1	18	19	1	92

Table 3.6 Marking qualities of female to male transmission of yellow fluorescent pigment powder on 4 subsequent days by brief contact.

day after initially marking	no.of females mated	unmarked	very poorly marked	poorly marked	well marked	very well marked	% of males marked
-	30	-	-	-	9	21	100
1	26	-	-	9	6	11	100
2	20	-	-	5	8	7	100
3	18	-	-	9	5	4	100

Table 3.7 Overall results of field experiments on contact events between tsetse flies using fluorescent pigment powder.

field experiment	I	II	III	IV	V
released marked flies	125 males	125 males	62 males	125 males	125 females
no. / % of recaptured originally marked flies	32 / 25.6	32 / 25.6	7 / 11.3	21 / 20.8	16 / 12.8
no.of captured originally unmarked females	29	108	7	75	110
no.of captured originally unmarked males	64	200	5	152	218
apparent pop.density in flies per trap and day including recaptured flies	25	68	3.8	82.7	68.8
sex ratio (female / male) including recaptured flies	0.3	0.47	0.58	0.43	0.58
no. / % of originally unmarked females which 'were contacted'	6 / 20.7	26 / 24.1	0	18 / 24.0	11 / 10.0
no. and % of originally unmarked males which 'were contacted'	12 /18.8	31 / 15.5	0	36 / 23.7	14 / 6.4
no. and % of originally unmarked females which 'have contacted'	not noted	1 / 0.9	0	0	1 / 0.9
no. and % of originally unmarked males which 'have contacted'	not noted	19 / 9.5	0	6 / 3.9	50 / 22.9

Table 3.8 P-values obtained when the pooled number of females and males respectively of experiments I, II, IV and V of each wing-fray category, which 'were contacted' and which 'were not contacted', was compared with the numbers of each other wing-fray category.

males\females	1T	1NT	2	3	4	5	6
1T	X	0.082	0.094	0.190	0.592	0.203	-
1NT	0.331	X	0.947	0.792	0.272	0.360	-
2	0.011	0.009	X	0.768	0.284	0.367	-
3	0.139	0.359	0.159	X	0.456	0.340	-
4	0.092	0.214	0.264	0.780	X	0.259	-
5	0.151	0.332	0.984	0.474	0.573	X	-
6	0.642	0.949	0.491	0.834	0.759	0.555	X

Table 3.9 P-values obtained when the pooled number of males and females of experiments I, II, IV and V of each wing-fray category, which 'were contacted' and which 'were not contacted', was compared with the pooled number of each other wing-fray category of the opposite sex.

	1T male	1NT male	2 male	3 male	4 male	5 male	6 male
1T female	0.005	0.008	0.329	0.054	0.085	0.366	0.271
1NT female	0.077	0.164	0.256	0.734	0.964	0.754	0,746
2 female	0.112	0.276	0.293	0.815	0.981	0.735	0.768
3 female	0.086	0.205	0.549	0.605	0.772	0.903	0.675
4 female	0.022	0.044	0.745	0.193	0.271	0.645	0.425
5 female	0.555	0.448	0.283	0.386	0.364	0.331	0.464
6 female	-	-	-	-	-	-	-

Table 3.10 P-values obtained when the pooled number of males of experiments II, IV and V of each wing-fray category, which 'have contacted' and which 'have not contacted', was compared with the number of each other wing- fray category.

	1T	1NT	2	3	4	5	6
1T	X	0.044	0.002	0.000	0.000	0.000	-
1NT	-	X	0.017	0.000	0.000	0.001	0.428
2	-	-	X	0.015	0.250	0.066	0.186
3	-	-	-	X	0.210	0.601	0.049
4	-	-	-	-	X	0.232	0.104
5	-	-	-	-	-	X	0.040
6	-	-	-	-	-	-	X

3.5 References cited

- Brady, J. (1972). The visual responsiveness of the tsetse fly *Glossina morsitans* Westw. (Glossinidae) to moving objects: the effects of hunger, sex, host odour and stimulus characteristics. *Bulletin of Entomological Research* 62: 257 - 279
- Bursell, E. (1961). The behaviour of tsetse flies in relation to problems of sampling (*G. swynnertoni* Austen). *Proc. R.ent. Soc. Lond.*(A) 36, 9-20
- Carlson, D.A.; Langley, P.A.; Huyton, P. (1978). Sex pheromone of the tsetse fly: isolation, identification and synthesis of contact aphrodisiacs. *Science* 201, 750-753
- Challier, A. (1982). Mini-Review. The ecology of tsetse (*Glossina* spp.) (Diptera, Glossinidae): a review (1970 - 1981). *Insect Science and its Application* 3 (2): 97 - 143
- Dame, D.A.; Ford, H.R. (1968). Multiple mating of *Glossina morsitans* Westw. and its potential effect on the sterile male technique. *Bulletin of Entomological Research* 58: 213-219
- Davies-Cole, J.A.O. (1990). Aspects of the mating behaviour of *Glossina morsitans morsitans* Westwood and *Glossina pallidipes* Austen. *PHD - thesis* Univ. of Sierra Leone
- Dean G., Clements S., Paget J. (1969). Observations on sex attraction and mating behaviour of the tsetse fly, *Glossina morsitans orientalis*. *Bulletin of Entomological Research* 59, 355-365

- Hall, M.J.R.; Langley, P.A. *(1989). The responses of individual males in an* isolated population of *Glossina morsitans morsitans* Westwood (Diptera: Glossinidae) to pheromone-baited decoy 'females'. *Bulletin of Entomological Research* 79: 319 - 334
- Harley, J.M.B. (1966). Studies on age and trypanosome infection rate in females of *Glossina pallidipes* Aust., *G. palpalis fuscipes* Newst. and *G. brevipalpis* Newst. in Uganda. *Bull. of Entomological Research* 57: 23 - 37
- Huyton, P.M.; Langley, P.A.; Carlson, D.A ; Coates, T.W. (1980a). The role of sex pheromones in initiation of copulatory behaviour by male tsetse flies, *Glossina morsitans morsitans. Physiological Entomology* 5, 243-252
- Huyton, P.M.; Langley, P.A.; Carlson, D.A.; Schwarz, M. (1980b). Specificity of contact sex pheromones in tsetse flies, *Glossina* spp. *Physiological Entomology* 5, 253-264
- Jackson C.H.N. (1946). An artificially isolated generation of tsetse flies (Diptera). *Bulletin of Entomological Research* 37: 291-299
- Jaenson, T.G.T. (1979a). Mating behaviour of males of *Glossina pallidipes* Austen (Diptera: Glossinidae). *Bull. of Entomol. Research* 69, 573-588
- Jaenson, T.G.T. (1979b). Mating behaviour of *Glossina pallidipes (*Diptera, Glossinidae): duration of copulation, insemination and fecundity. *Ent. exp. and appl.* 26, 1-12
- Jaenson, T.G.T. (1980). Mating behaviour of females of *Glossina pallidipes* Austen (Diptera: Glossinidae). *Bull. of Entomological Research* 70, 49-60

- Langley P.A., Pimley R.W., Carlson D.A. (1975). Sex recognition pheromone in tsetse fly *Glossina morsitans. Nature* 254, 51-53
- Langley, P.A. (1977). Physiology of tsetse flies (*Glossina* spp.) (*Diptera: Glossinidae)*: a review. *Bulletin of Entomologiacl Research* 67, 523-574
- Langley, P.A; Hall, M.J.R. (1984). Aspects of the regulation of mating behaviour in tsetse. *Insect Science and its Application* 5(5): 351 - 355
- Langley, P.A.; Huyton, P.M.; Carlson, D.A. (1987). Sex pheromone perception by males of the tsetse fly, *Glossina morsitans morsitans. Physiological Entomology* 12, 425-433
- Masner, P.; Slama, K.; Landa, V. (1968). Sexually spread insect sterility induced by the analogues of juvenile hormone. *Nature, London* 219: 395 – 396
- Mwangelwa, M.I. (1992). The ecology and vectorial capacity of *Glossina fuscipes fuscipes* on Rusinga Island along the shore of Lake Victoria. *PhD-thesis*. University of Zambia.
- Nash T.A.M. (1955). The fertilisation of *Glossina palpalis* in captivity. *Bulletin of Entomological Research* 46, 357-368
- Nash, T.A.M.; Jordan, A.M.; Boyle, J. (1968). The large-scale rearing of *Glossina austeni* Newst. In the laboratory. IV. The final technique. *Annals of Tropical Medicine and Parasitilogy* 62, 336-341
- Rogers, A. (1973a). Multiple mating by male *Glossina pallidipes* in the laboratory. *Trans.R.Soc.trop.Med.Hyg.* 67, 298-299

- Rogers, A. (1973b). The breeding of *Glossina pallidipes* Austen (Diptera: Glossinidae) in the laboratory and in the field. - 244pp. *Ph.D. - thesis*, Univ.Salford.
- Saunders, D.S. (1962). Age determination for female tsetse flies and the age composition of samples of *G. pallidipes, G. palpalis fuscipes* and *G. brevipalpis*. *Bulletin of Entomological Research* 53: 579 - 595
- Swynnerton, C.F.M. (1936). The tsetse flies of East Africa. A first study of their ecology, with a view to their control. *Transactions of the Royal Entomological Society of London* 84: 1-579
- Turner D.A. (1971). Olfactory perception of live hosts and CO_2 by the tsetse fly, *Glossina morsitans orientalis. Bulletin of Entomological Research* 61, 75-96
- Turner, D.A. (1980). A novel marking/release/recapture method for possible use in determining aspects of tsetse fly behaviour. *Insect Science and its Application* 1: 1-7
- Vale, G.A. (1974). The response of tsetse flies (Diptera, Glossinidae) to mobile and stationary baits. *Bulletin of Entomological Research* 64, 545-588
- Vanderplank, F.L. (1947). Experiments in the hybridisation of tsetse flies (Glossina, Diptera) and the possibility of a new method of control. *Trans. R.ent. Soc. Lond.* 98, 1-18
- Wall, R. (1989). The roles of vision and olfaction in mate location by the tsetse fly *Glossina morsitans morsitans. Medical and Veterinary Entomology* 3: 147 – 152

CHAPTER FOUR

CONTROL EFFECT OF THE ENTOMOPATHOGENIC FUNGUS *METARHIZIUM ANISOPLIAE* ON A POPULATION OF *GLOSSINA FUSCIPES FUSCIPES*

4.1 Introduction

It has been shown in recent years that trapping can effectively control tsetse flies (Vale *et al.*, 1986; Brightwell *et al.*, 1987; Dransfield *et al.*, 1990). The method is environmentally safe, adaptable in rural areas and can be accomplished by local communities themselves (Wall and Langley, 1991). However, there are limitations to this method including the availability of adequate number of traps, theft, natural damage and loss of traps due to bush-fire or seasonal floods. Moreover, traps have to be served regularly because of rapid vegetation growth, which reduces the visibility. In addition, traps are of limited duration due to the material used (Laveissiere, 1990). That is why, there is need to increase the efficiency of a trap in order to reduce their number. That could be achieved with the 'Dissemination Technique' (Masner *et al.*, 1968). The concept is based on passing a sterilising or lethal agent from contaminated released insects to other target-insects through contact events. One of the promising agents is the entomopathogenic fungus *M. anisopliae*. The fungus can cause high mortality rates up to 100% (Kaaya and Okech, 1990). Captured wild tsetse flies can be contaminated by passing through a contamination device (CD) mounted on a trap, and can then be released automatically. Various CD's were developed for tsetse control (House, 1982;

Langley, 1995; Maniania, 1998). Maniania (1998) tested several types of CD's which were contaminated with dry conidia of *M. anisopliae* and mounted on biconical traps and reported mortality rates of up to 86 %. Thereafter, a further improved CD caused infection rates of 100 % in semi-field trials (Maniania, 1998 pers.comm.). The objective of this study was to determine the efficiency of the 'Dissemination Technique' using Maniania's CD contaminated with dry conidia of *M. anisopliae* as an alternative to trapping. This study was carried out with *G. fuscipes fuscipes* in a thicket on the shores of Lake Victoria and on a small island in Lake Victoria in Suba District, Kenya.

4.2 Materials and methods

4.2.1 Estimation of the control efficiency

The control effect of the contaminated CD's was estimated using the software 'Model works'. In order to simplify the model, only the female population were taken into account. The model was based on the initial number of females, the proportion of females passing daily the CD's, the natural mortality and natality rate, the lethal time to kill 50% of the infected females (LT_{50}) and the pupal duration. The output of the model was data on the absolute number of females and of the infection rate of the entire female population at any day after the exposure of the contaminated CD's.

The inputs of the model were obtained as follows:

- *Initial number of females*: Prior to the control attempt, a capture-mark-recapture-study was carried out.

- *Proportion of flies passing daily the CD's:* During the control attempt, the same biconical type of traps and the same trap positions as during the capture-mark-recapture-study were used. Additionally, the capture probability of a fly was assumed to be constant at different population densities. Therefore, it was possible to calculated the number of flies theoretically passing through the CD's every day.
- *Natural mortality and natality rate:* The natural mortality rate of the population was estimated according to Challier and Turner (1985) prior to the exposure of the contaminated CD's using data on the age structure in May and June. The natality rate was assumed to be as high as the mortality rate since the population appeared stable during that time. The method is based on a graded age structure and the duration of the interlarval period. The underlying assumptions of the method are that the mortality rate of each ovarian age category is constant and that the population is stable. The ovarian age category 0 was omitted as this group was considered to be undersampled by traps (Challier, 1982), which would otherwise result in an under-estimated mortality rate. Both the natural mortality and natality were assumed not to change during the control attempt. The larval period was assumed to be 9 days as it was reported for the study area (Mwangelwa, 1992).
- *LT_{50}:* Contaminated with dry conidia of *M. anisopliae,* a similar type of Maniania's CD caused a LT_{50} of about 5.6 and 9.5 days when *G. pallidipes / G. longipennis* passed the CD on the day of exposure and 21 days after

initial exposure to field conditions (Maniania, 1998). The LT_{50} of the present model was assumed to be 10 days.

- *Pupal period:* According to Symes and Southby (1938), *G. fuscipes fuscipes* has a pupal period of 43-53 days under natural conditions on the Kenyan shore of Lake Victoria. Therefore, the mean duration of the pupal period was assumed to be 48 days.

Further assumptions were made:

- The natural population density would remain constant during the control attempt.
- The infected flies reproduced normally until death.
- Due to logistical reasons, it was impossible to determine the absolute population density but only the apparent population density on monthly basis. Furthermore, due to the way of capturing and handling the flies, mark-release-recapture studies could have caused an artificial increased infection rate of the population when carried out during the exposure of the contaminated CD's. In order to compare the absolute number of females of the following months with the model, it was assumed that the absolute and apparent population density were positively linear correlated. Therefore, the absolute number of females obtained from the mark-release-recapture study (absolute population density) and the number of females per trap and day obtained from the subsequent monitoring (apparent population density)

in June were considered to represent the same situation and those data used as standard reference.

- The contaminated CD's and the control traps had to be removed during the monthly monitoring to exclude an interference between monitor and control traps. Therefore, it was assumed that the contaminated CD's were applied continuously until the end of the experiment.
- No infection through contact events took place resulting in an estimation of the maximum infection rate of the CD when fitting data on the observed final apparent population density and data on the observed infection rate of the entire population.

The model was used to estimate the following:

1) The effect of the contaminated CD's on the number of those females, which had emerged up to the exposure of the contaminated CD's, when the infection rate of the CD was 100 %.
2) The effect of the contaminated CD's on the number and the infection rate of all females when the infection rate of the CD was 100 %.
3) The maximum infection rate of the CD, when fitting the data on the observed final apparent population density.
4) The maximum infection rate of the CD when fitting the data on the observed infection rate of the entire population.

4.2.2 Monitoring of the apparent population density

The northern Chamaunga Island was chosen to provide data on the control effect of the contaminated CD's. The Kisui-thicket was chosen as control to provide data on the natural development of a population of *G. fuscipes fuscipes* in that area assuming that the biotic and abiotic factors affected both populations in the same way. Therefore, the apparent population density of both sites was monitored for three days monthly from March 1998 to September 1998. On the northern Chamaunga Island, thirteen pyramidal traps were placed in three groups. Two groups contained five traps each, which were placed along the shore at a distance of about 30 m between each other. The other three traps, were placed inside the island 30 m between each other. In the Kisui-thicket, twelve pyramidal traps were placed in three groups (2, 5, 5) along the shore at a distance of about 30 m between each trap. The cages were changed after 24 hours. All flies were sexed and counted at MPFS.

4.2.3 Ageing of flies

The objective was to compare the development of the age structures of both populations. Therefore, each month from March 1998 to September 1998 between 50 and 95 randomly chosen captured females of each site were aged using the ovarian age method (Saunders, 1962). Freshly killed flies were examined on the day of capture or on the next day using a dissecting microscope.

4.2.4 Mark-release-recapture study

The objective of the mark-release-recapture study was to determine the total number of flies occurring prior to the control attempt on the northern Chamaunga Island. Therefore, for three days, nine traps were placed along the shore at a distance of approximately 85 m from each other and three traps inside the island with a distance of approximately 70 – 80 m from each other and from the shore. Six assistants served 1-2 traps each emptying the cages at least every hour. The flies were marked with small dots of acrylic dye on the dorsal thorax (1st day - green, 2nd day - red, 3rd day - yellow) and thereafter immediately released. All recaptured flies were noted according to their sex, after which they were immediately released to avoid an artificial population decline. When recaptured the same day, they were immediately released without remarking. When recaptured on any following day, they were remarked using another colour (2nd day - white, 3rd day - yellow). That was in order to recognise on the third day a fly which was captured on the first as well as on the second day. Therefore, such a fly bore a green and a white dot and had to be included to the number of flies which bore a red dot. Moreover, a recount of the same fly was therewith avoided when recaptured twice or more often on the following day. The two recapture days were considered as two separate experiments (A, B).

The absolute population density was estimated according to the Lincoln-Index $N = m\,c / r$ (Otis *et al.*, 1978). N is the estimated total number, m the number of marked individuals on day of marking, c the number of all captured individuals

on day of recapture and r the number of marked individuals on day of recapture.

4.2.5 Maniania's contamination device

Maniania's CD (Fig. 11) consisted of a plastic bottle, net and velvet material and three wires. Transparent net material was fixed on the entire length between two parts of a plastic bottle dividing it into two parts to narrow the space. One hole each was cut out near the bottom and near the top on the same side of the bottle. Being an excellent material for the conidia to stick on, non-transparent velvet material was lined in one half of the divided bottle except around and above both cut holes in order to use up the positive phototaxis of tsetse flies to enter and leave the CD. One wire surrounded with velvet material was fixed along the inside of the bottle to narrow the space further. The top of the bottle was closed with a cover. The CD was mounted and fixed with two wires on a trap rising about 45° from the horizontal (Photo 3). The flies were supposed to enter the CD through the hole near the bottom and to leave automatically through the hole near the top.

Plate 4.1 Maniania's contamination device mounted on a biconical trap.

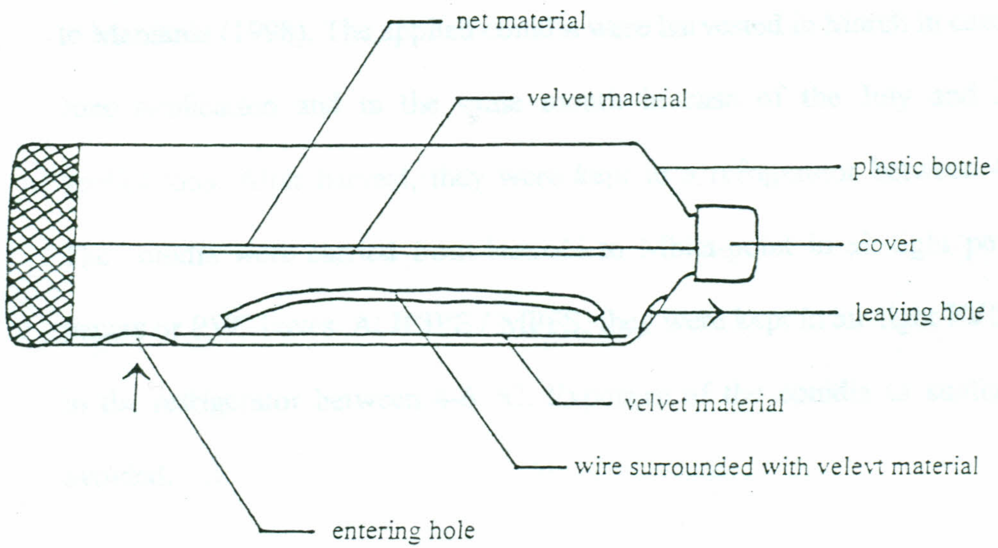


Figure 4.1 Sketch of Maniania's contamination device.

4.2.6 Fungus application

The control attempt started the following day after the monthly monitoring in June and lasted for three months. The control traps had the same positions as during the mark-release-recapture study and were also from the same biconical type. Each trap was mounted with a contaminated CD. About two grams of dry conidia of the strain IC 30 of *M. anisopliae* were applied into each CD. On a weekly basis, the vegetation surrounding the traps was cut back and dead flies counted which were found inside the traps and CD's. In order to carry out the monthly monitoring of the apparent population density, the control traps were removed and replaced at the end of the 3-day-monitoring. With each replacement, the remaining conidia of the previous period were removed, and the same CD's were contaminated with two grams of new conidia each .

At ICIPE Nairobi, the conidia were mass-produced on maize medium similar to Maniania (1998). The applied conidia were harvested in March in case of the June application and in the same month in case of the July and August applications. After harvest, they were kept in a refrigerator between 4-6 °C. The conidia were carried from Nairobi to Mbita-point in air-tight polythene papers or PVC boxes. At ICIPE / MPFS, they were kept in air-tight PVC boxes in the refrigerator between 4-6 °C. Exposure of the conidia to sunlight was avoided.

4.2.7 Germination rate of the conidia

The germination rates were tested before and after each application using a Malaise Counting Chamber. After each application, three samples of different CD's were tested and the results averaged.

4.2.8 Determination of the infection rate of the tsetse population

The objective was to monitor the infection rates of both populations with *M. anisopliae* during the exposure of the contaminated CD's. Therefore, flies of both populations were obtained on monthly basis during the regular monitoring.

On the northern Chamaunga Island, 100 flies were captured individually on three traps. Each trap was served by one assistant who was hidden about 10 m from the corresponding trap. Each captured fly was transferred from the cage into a small PVC box having a net cover (height 35 - 40 mm, diameter 26 - 31 mm) using a sucking tube (glass pipe or piece of water hose, length 270 - 280 mm, inner diameter 9 - 13 mm) and a small piece of textile. Several measures were used in order to avoid artificial transmission of conidia between the flies. Therefore, with each captured fly, the cage frame, the net covering the cage frame, the sucking tube and the piece of textile were changed while having a sufficient number of them at each trap. The captured flies were fed still in the field and again in the evening on a live goat.

Furthermore, 100 flies each from the northern Chamaunga Island and the Kisui-thicket which were captured not individually were kept starting with 20 flies per PVC cage used for rearing. This was to determine whether the capture procedure and the keeping condition had a significant effect on the infection rate. It was further aimed at excluding an effect of *M. anisopliae* on the population of the Kisui-thicket.

The captured flies were fed daily on a life goat until they died. Using a disinfected tweezers, the dead flies were placed in a petri dish on a filter paper which was soaked in distilled water. The petri dish was then airtight closed with parafilm. In that water saturated atmosphere, the fungus would grow saprophytically developing conidiospores on the cuticle surface (Ferron, 1981). *M. anisopliae* was detected by the characteristic growth form and characteristically colour using a dissecting microscope and by the size and characteristic oval shape of the conidia using a compound microscope. Since other saprophytic fungi can overgrow *M. anisopliae,* suspicious looking but not obviously infected flies were examined for the conidia.

4.2.9 Statistical analysis

The infection rates were compared using the chi square test using SPSS 6.12 software.

4.3 Results

4.3.1 Apparent population density

Prior to the control attempt, the apparent population density in the Kisui-thicket and on the northern Chamaunga Island fluctuated approximately in the same way. It almost doubled from March to May and decreased slightly in June (Fig. 4.2). During the exposure of the contaminated CD's from June to September, the apparent population density on the northern Chamaunga Island declined to about one third (Fig. 4.2a). On the other hand, the apparent population density in the Kisui-thicket increased in July and August but then decreased in September to about the same level as in June (Fig. 4.2b).

4.3.2 Sex-ratio

Generally, the sex-ratio of both sites fluctuated almost equally. It fluctuated around 1 on the northern Chamaunga Island while in the Kisui-thicket mostly above 2 showing considerably more females (Fig. 4.3).

4.3.3 Age structure

The age structure of the female population of the northern Chamaunga Island remained relatively constant from March to June. During the control attempt, the proproportion of younger females clearly increased from June to September (Fig. 4.4). The age structure of the female population of the Kisui-thicket over the whole period showed a slight change to more younger flies from May to June (Fig. 4.5). However, during the control attempt, the age structure of females from the Kisui-thicket remained approximately the same.

4.3.4 Mark-release-recapture study

During the study, a total of 3,311 flies were marked. When both experiments (A, B) were considered together, it was calculated that a total of 6,556 flies (3,257 females and 3,299 males) occurred in June prior to the exposure of the contaminated CD's on the northern Chamaunga Island (Table 4.1). This corresponded to an absolute population density of about 88,000 females and 89,000 males per km^2 or 880 females and 890 males per ha. On the following day of marking, the recapture rates were 16.7 and 22.2 % for females and 22.1 and 20.9 % for males. In average, 628 females and 699 males were daily captured which was 19.3 and 21.2 % of the estimated absolute number.

4.3.5 Germination rate of conidia

Before each application, germination rates of conidia were found to be between 89.5 and 91 % and afterwards between 8.6 and 14.5 %.

4.3.6 Infection rate of the tsetse population

There was no significant difference ($P > 0.05$) between the infection rates of males and females and between the infection rates when flies were captured and kept individually and together. The infection rates were 17.8 and 11.3 % for the monitoring in July and August on the northern Chamaunga Island. Data for September could not be obtained due to logistical problems. The average of available data was therefore 14.55 %. No fly was found to be infected in the Kisui-thicket.

4.3.7 Estimation of the control efficiency

The model was based on an initial number of 3,257 females, a proportion of 19.3 % of the females passing daily through the CD's, a natural mortality and natality rate of 2 %, a LT_{50} of 10 days and a pupal period of 48 days.

The control revealed that:

1) all females, which had emerged up to the start of the CD's exposure in June, were dead by day 47 assuming that the infection rate of the CD was 100 %. After one month, about 25 of those females were still alive corresponding to an apparent population density of about zero (Fig. 4.6a).
2) the apparent population density declined within three months from 20.7 females per trap and day (~ 3,250 females) to 1.5 females per trap and day (~ 240 females) assuming that the infection rate of the CD was 100 %. The infection rate of the whole population was around 80 % remaining approximately constant from day 9 (Fig. 4.6b). When all flies passing the CD's were infected, the population density declined in steps (Fig. 4.6b). Firstly, the adults which had emerged up to the start of the experiment started dying from day 10 after exposure of the contaminated CD's. That resulted in a sharp drop. Thereafter, the number remained approximately constant from day 20. That was because the number of emerging flies was similar to the number of dying flies due to the delay of death after infection. The next decline started at day 58 when the number of emerging flies begun to drop. That was because the number of larvidepositions began

to drop from day 10 and the pupal period was 48 days. As control became less effective these steps disappeared (Fig. 4.7).

3) when fitting the observed final apparent population density of 7.9 females per trap and day (~ 1,240 females), only 3.2 % but not 19.3 % of the population was contaminated daily suggesting that the maximum infection rate of the CD was about 16.6 % (Fig. 4.7a).
4) when fitting the observed infection rate of the entire population of 14.55 %, only 2 % but not 19.3 % of the population was contaminated daily suggesting that the maximum infection rate of the CD was about 10.4 % (Fig. 4.7b).

4.4 Discussion

Prior to the exposure of contaminated CD's, the apparent population density in the Kisui-thicket and on the northern Chamaunga Island fluctuated approximately in the same way suggesting that the Kisui-thicket can be considered as a control. Furthermore, it was shown that the population in the Kisui-thicket was not affected by *M. anisopliae.* At the beginning as well as at the end of the exposure of the contaminated CD's on the northern Chamaunga Island, there was approximately the same apparent population density in the Kisui-thicket. That suggested that the apparent population density would have remained approximately constant also on the northern Chamaunga Island being one of the assumptions for the model.

Males were reported to be more susceptible to the fungus than females (Kaaya, 1989; Kaaya and Okech, 1990). However, Maniania (1994) stated that both males and females are equally susceptible. In this study, there was no significant difference between infection rates of males and females obtained from the field suggesting that both sexes are equally susceptible to the fungus. Furthermore, the sex-ratio fluctuated almost equally prior to and during the exposure of the contaminated CD's.

It seems not necessary to capture and keep tsetse flies individually when investigating the infection rate of a tsetse population with *M. anisopliae*. Therefore, handling time and costs can be reduced when flies are captured and kept together.

The aim of the 'Dissemination Technique' is to increase the control effect of a trap. That means, the 'Dissemination Technique' must kill more flies than trapped. Moreover, the 'Dissemination technique' may have to cause the morefold of mortality. That is in order to compensate disadvantages of that technique. This includs the delay of death after passing a CD, additional larvipositions of the infected females which would be avoided by trapping and additional costs through the mass production and regular application of any agent. In order to increase the efficiency of trap with this technique, there must be a high infection rate of the CD and a high number of contact events between flies successfully infecting healthy flies.

After the exposure of the contaminated CD's, the apparent population density on the northern Chamaunga Island declined within three months to about one third. Moreover, the proportion of young females increased considerably. According to data on the control site, both the apparent population density and the age structure should have been approximately the same at the end as well as at the beginning of the exposure of the contaminated CD's, showing that the population on the northern Chamaunga Island was affected by the CD's.

However, the model revealed that less flies were killed during the exposure of the contaminated CD's than an infection rate of 100 % of the CD would have achieved. For this, it was shown that after one month the number of those females, which had emerged up to exposure of contaminated CD's, should have been close to zero. In contrast, data on the July monitoring showed a

proportion of about 36 % in ovarian age categories 4-7 corresponding to about 660 females older than 30 days. Furthermore, after three months, the apparent population density should have been about 1.5 females per trap and day. In contrast, the observed apparent population density was 7.9 females per trap and day after three months. Moreover, the infection rate of the entire population should have been about 80 % from day 9. In contrast, one and two month after the exposure of the contaminated CD's, the observed infection rate of the entire population was about 17.8 and 11.3 % respectively.

Fitting the data on the observed infection rate of the entire population and the observed final apparent population density, the contaminated CD's caused an estimated maximum infection rate of 10.4 and 16.6 % respectively. Provided that infections through contact events occurred, the actual primary infection rate of the CD was even lower than that. This is because, the parameters of the present model took only the infections through the CD's into account. However, the observed infection rate of the entire population and the observed decline of the apparent population density used for the estimation were determined by the combination of both the infection through the CD and the infection through contact events. Therefore, the estimations included to a certain extent the effect of contact events resulting in an estimation of the maximum infection rate of the CD.

According to the data on the control site, the decline of the population had completely an artificial cause. However, this decline might have not been

caused only by the fungus, because the maximum infection rate of the CD was estimated to be 16.6 % when fitted to data on the observed final apparent population density, but only 10.4 % when fitted to data on the observed infection rate of the entire population. Therefore, the CD's might have had another effect. It was observed that a number of flies were reluctant to enter and to leave the CD. However, no data was available on how long they stayed within the trap before entering the CD, how long they stayed in the CD before leaving it and which proportion of flies finally passed the CD. In the three subsequent months of exposure of the contaminated CD's, a total of 113, 46 and 27 dead flies respectively were found in the traps and the CD's. About one third of them were found in the CD's and two third in the traps, with no sex being predominant. This suggested that more flies could have left the trap without entering the CD but did not survive since they were getting weak. Furthermore, a number of flies, which passed the CD, might have died shortly afterwards because they lost too much energy while being in the trap and CD. Indeed, the observed decline of the population density might have been caused fully by the CD, and, not due to the fungus alone. The 16.6 % maximum infection rate of the CD, estimated when fitting the data on the observed final apparent population density, may therefore include the effect of the reluctance of the flies to enter and leave the CD's.

Therefore, the actual maximum infection rate of the CD could have been higher than 10.4 %, estimated when fitting the data on the infection rate of the entire population. This is because less flies than assumed for the model might

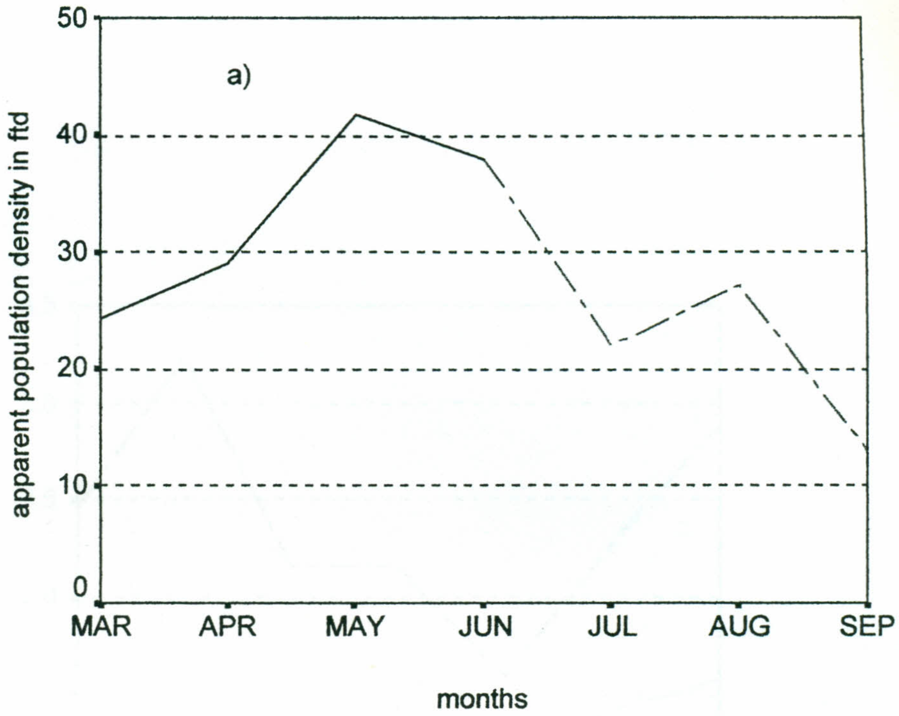
have gone daily through the CD, which would have lead finally to an underestimation of the CD's maximum infection rate. However, during observations, it was estimated that at least 50 % of those flies, which entered the trap, finally passed the CD while still having enough energy to survive. According to the model, if only half the number of flies passed daily through the CD's (9.65 %) then the maximum infection rate of the CD was about 20 % when fitting to the 14.6 % infection rate of the entire population. Therefore, the maximum infection rate of the CD was between 10 and 20 %.

There is evidence that the absolute and apparent population densitiy are positively linear correlated as found with a population of *G. palpalis gambiensis* (Cuisance *et al.*, 1978, 1980), a close relative to *G. fuscipes fuscipes*. This would meet the assumption for the model. Other studies revealed an inverse relationship between catch index and population density for *G. fuscipes fuscipes* (Mwangelwa, 1990; Gouteux *et al.*, 1995; Muhigwa *et al.*, 1998). Moreover, Muhigwa (1998) has shown that the less dead flies were stacked on a trap the more the females entered it. That means, the proportion of flies which passed daily through the CD's might have been higher than assumed at declining population density resulting in an overestimation of the CD's effect. Also, the chosen LT_{50} was more likely too high, the chosen pupal period too long and the reproduction might have been affected by an infection leading again to an overestimation of the CD's effect and leaving in that way no doubt about its inefficiency. Additionally, in order to simplify the model, it was assumed that the 3 days monthly monitoring had the same effect as the

contaminated CD's. However, according to the results, the monitoring had probably a higher effect than the CD's leading once more to an overestimation of the CD's efficiency. Though, the difference of both effects was not easy to figure out because the positions of the 13 monitoring traps was different from the positions of the 12 control traps.

The 'Dissemination Technique' must kill more flies than trapped or as in this case passing through the CD's to be an alternative to trapping. However, during the exposure of the contaminated CD's, less flies than those passing through the CD's were killed. That was because the infection rate of the CD was clearly less than 20 % and the number of infections through contact events were too low to compensate that. Therefore, Maniania's CD contaminated with dry conidia of *M. anisopliae* would not be considered as an alternative to trapping.

Maniania's CD has to be modified to use better the positive phototaxis of tsetse flies in order to attract them faster into the CD and not to keep them inside the CD for too long, so that all flies entering the trap pass through the CD and that after passing trough the CD, the flies are still fit enough to transmit the conidia. Furthermore, the CD has to be improved with respect to the infection rate of passing flies and the germination rate of the applied conidia. The latter showed within one month of exposure to natural conditions a decline from around 90 to 12 %.



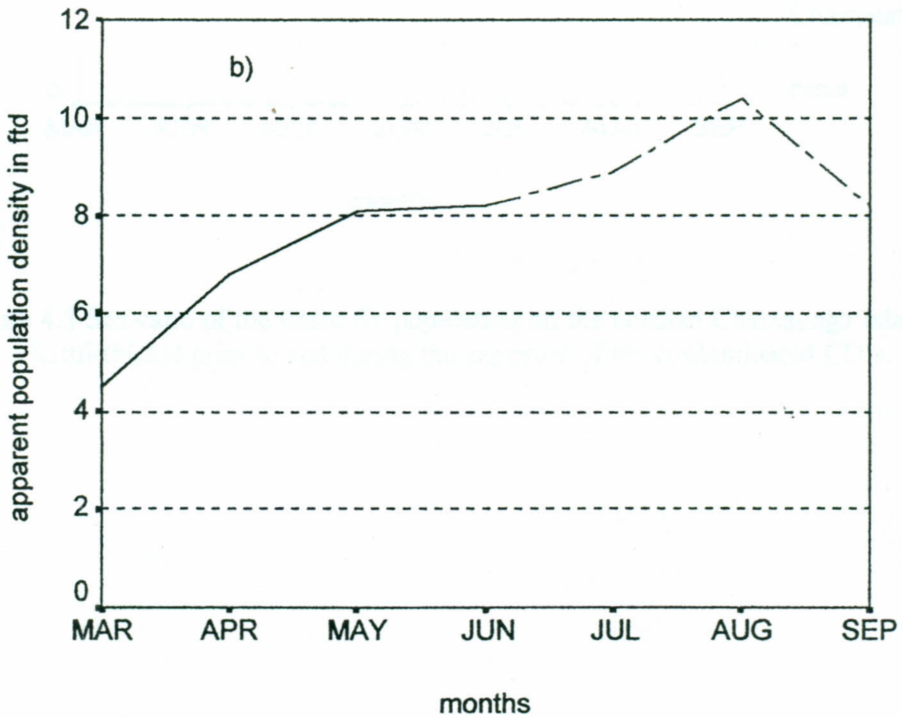


Figure 4.2 Apparent population density in ftd (flies per trap and day) a) of the northern Chamaunga Island and b) of the Kisui-thicket from March '98 to September '98 (— up to exposure of contaminated CD's on the northern Chamaunga Island, – · – · – during exposure of contaminated CD's on the northern Chamaunga Island).

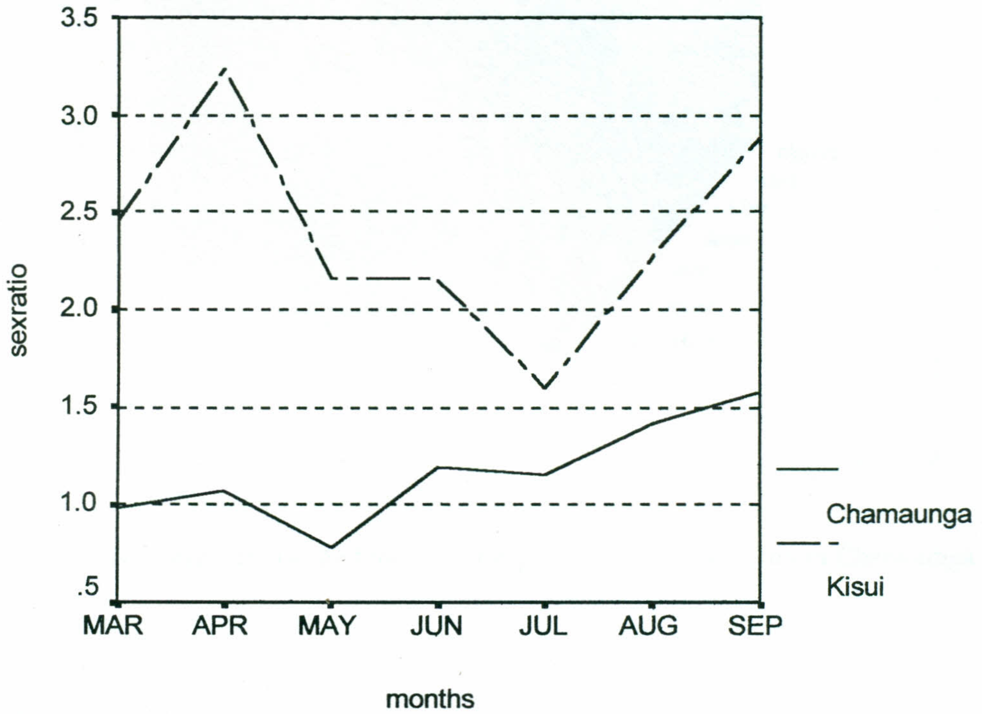


Figure 4.3 Sex-ratio of the tsetse fly population on the northen Chamaunga Island and in the Kisui-thicket prior to and during the exposure of the contaminated CD's.

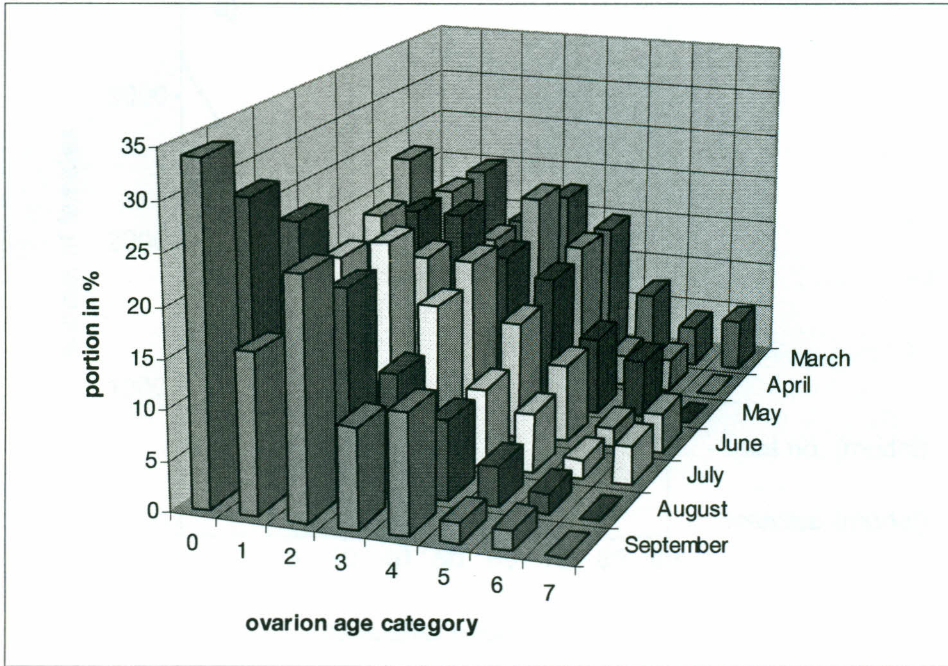


Figure 4.4 Age structure of the female tsetse population of the northern Chamaunga Island.

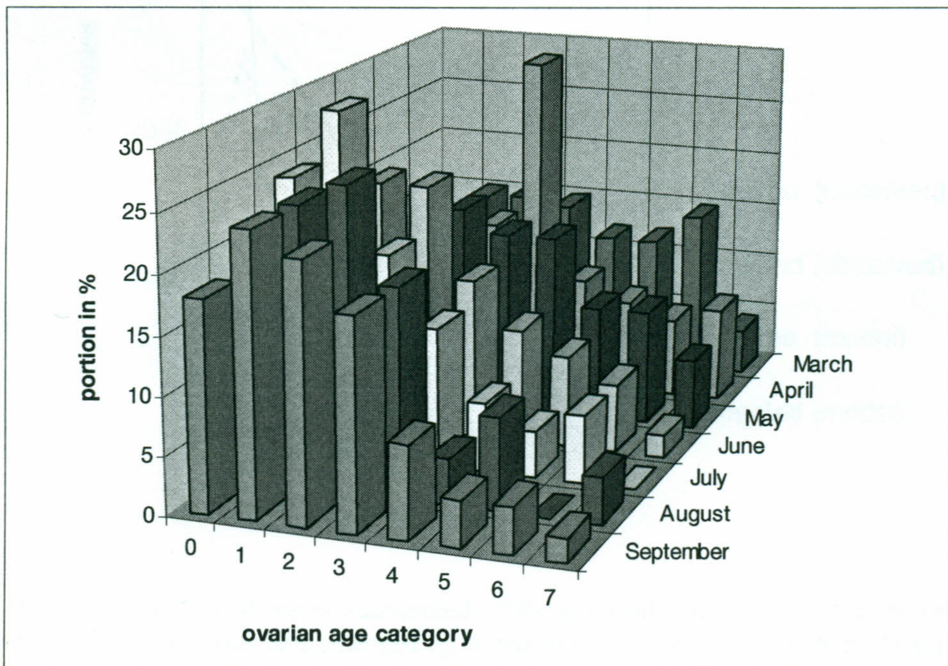


Figure 4.5 Age structure of the female tsetse population of the Kisui-thicket.

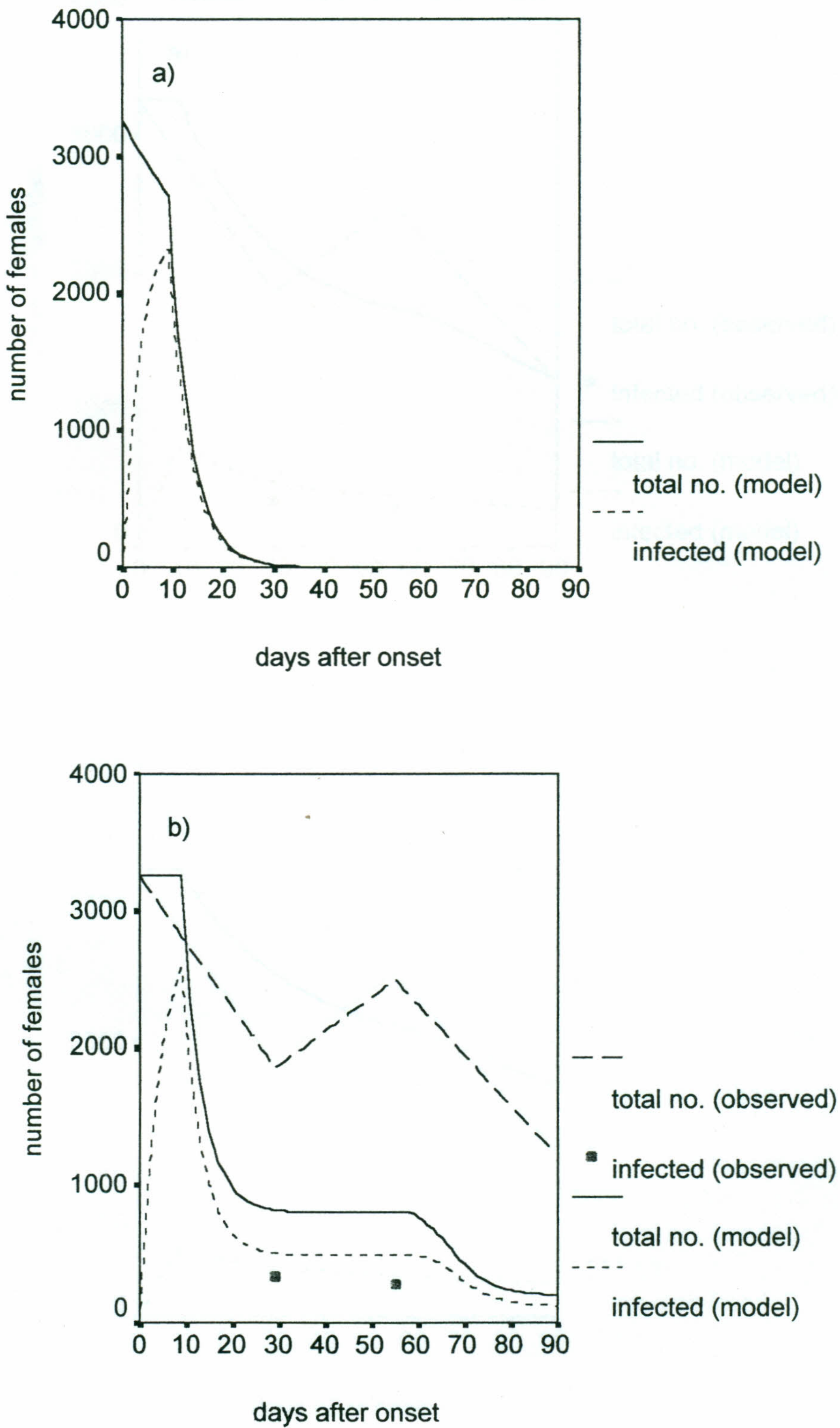
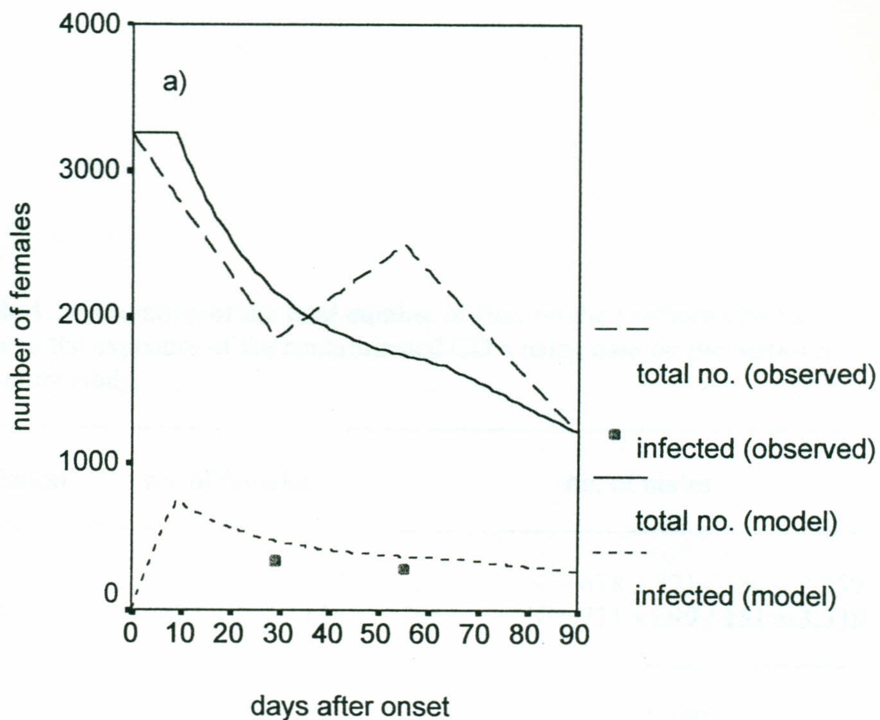
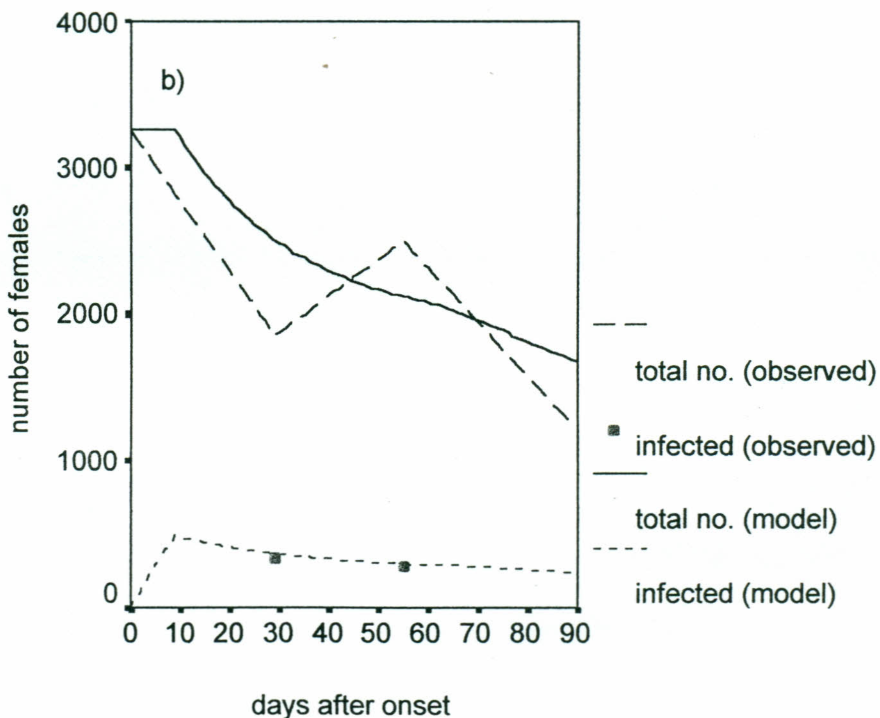


Figure 4.6 Effect of the contaminated CD's at an infection rate of 100 % on a) only those females which had emerged up to the exposure of contaminated CD's and b) all females and the comparison with the observed field data.





Figures 4.7 Maximum infection rate of the CD being a) 16.6 % when fitting the data on the observed final apparent population density and b) 10.4 % when fitting the data on the observed infection rate of the tsetse population.

Table 4.1 Estimation of the total number of flies on the northern Chamaunga Island prior to the exposure of the contaminated CD's using data on the mark-release-recapture study.

repetition	no. of females	no. of males	sum
A	N = 641 x 621 / 107 = 3,720	N = 678 x 721 / 150 = 3,259	6,979
B	N = 621 x 621 / 138 = 2,794	N = 721 x 699 / 151 = 3,338	6,132
average	3,257	3,299	6,556

4.5 References cited

- Brightwell, R.; Dransfield, R.D.; Kyorku, C.; Golder, T.K.; Tarimo, S.A.; Mungai, D. (1987). A new trap for *Glossina pallidipes*. *Tropical Pest Management* 33: 151-159
- Challier, A. (1982). Mini-Review. The ecology of tsetse (*Glossina* spp.) (Diptera, Glossinidae): a review (1970 - 1981). *Insect Science and its Application* 3 (2): 97 - 143
- Challier, A.; Turner, D.A. (1985). Method to calculate survival rate in tsetse fly (*Glossina*) populations. *Ann. Soc. belge Mèd. trop.* 65: 191 - 197
- Cuisance, D.; Politzar, H.; Clair, M.; Sellin, E.; Taze, Y. (1978). Impact des lâchers de mâles stériles sur les niveaux populations sauvages de *Glossina palpalis gambiensis* en Haute-Volta (sources de la Volta Noire). *Revue Élev. Méd. Rét. Pays trop.* 31: 315 – 328
- Cuisance, D.; Politzar, H.; Fevrier, J.; Bourdoiseau, G.; Sellin, E. (1980). Association d'un traitement insecticide avec la méthode du mâle stérile contre *Glossina palpalis gambiensis*: intérèt de la mise en oeuvre de plusieurs méthodes: *Revue Élev. Méd. Vèt. Pays trop.* 33: 127 - 133
- Dransfield, R.D.; Brightwell R.; Chaudhury, T.K.; Golder, T.K.; Tarimo, S.A.R. (1986). The use if odor attractante for sampling *Glossina pallidipes* Austen (Diptera: Glossinidae) at Nuguruman, Kenya. *Bulletin of Entomological Research* 76: 607 - 619

- 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
- Ferron, P. (1981). Pest control by the fungi *Beauveria* and *Metarhizium*. In „*Microbial control of pests and plant diseases 1970-1980*" (H.D.Burges, Ed.), pp. 465-482. Academic Press, London.
- Gouteux, J.P.; Blanc, D.; Cuisance, F.D'Amico; Guinza A.K. (1995). Trials of olfactory attractants to enhance trap catches of *Glossina f. fuscipes* (Diptera: Glossinidae) in the Central African Republic. *Vet. Res.* 26: 335 - 340.
- House, A.P.R. (1982). Chemosterilisation of *Glossina morsitans morsitans* Westwood and *G.pallidipes* Austen (Diptera: Glossinidae) in the field. *Bulletin of Entomological Research* 72: 65 - 70
- Jackson, D.M.; Brown, G.C.; Nordin, G.L.; Johnson, D.W. (1992). Autodissemination of baculovirus for management of tobacco budworms (Lepidoptera: Noctuidae) on tobacco. *J. Econ. Entomol.* 85: 710 – 719
- Kaaya, G.P. (1989). *Glossina morsitans morsitans*: Mortalities caused in adults by experimental infection with entomopathogenic fungi. *Acta Tropica* 46: 107-114
- Kaaya, G.P.; Okech, M.A. (1990). Horizontal transmission of mycotic infection in adults tsetse, *Glossina morsitans morsitans*. *Entomophaga* 35(4): 589-600

- Langley, P.A. (1995). Evaluation of the chitin synthesis inhibitor triflumeron for controlling the tsetse *Glossina morsitans morsitans (*Diptera: Glossinidae*). Bulletin of Entomological Research* 85, 495-500
- Laveissiere, C. (1990). Sleeping sickness: peasants in charge of vector control. *International Scientific Council for Trypanosomiasis Research and Control. 20th meeting*, Mombasa. Kenya, 10-14th April 1989. Nairobi
- Maniania, N.K. (1994). A laboratory technique for infecting adult tsetse with fungal pathogen. *Insect Science and its Application* 15 (4/5): 421 - 426
- Maniania N.K. (1998). A device for infecting adult tsetse flies, *Glossina* spp., with an entomopathogenic fungus in the field. *Biological Control* 11, 248-254
- Masner, P.; Slama, K.; Landa, V. (1968). Sexually spread insect sterility induced by the analogues of juvenile hormone. *Nature, London* 219: 395 – 396
- Muhigwa, J.-B. B. (1998). *Glossina fuscipes fuscipes* Newstead 1910 (Diptera, Glossinidae): Colour preferences, host-finding strategies and responsiveness to baited traps. *PhD-thesis*, University of Khartoum
- Muhigwa, J.-B. B.; Saini, R.K.; Hassanali, A. (1998). Effects of fly abundance on catch index of traps for *Glossina fuscipes fuscipes* (Diptera: Glossinidae). *J. Med. Entomol.* 35 (2): 148 - 152
- Mwangelwa, M.I. (1992). The ecology and vectorial capacity of *Glossina fuscipes fuscipes* on Rusinga Island along the shore of Lake Victoria. *PhD-thesis*. University of Zambia.

- Otis, D.L.; Burnham, K.P.; White, G.C.; Anderson,D.R. (1978). Statistical inference from capture data on closed animal populations. *Wildlife Monographs* 43(62): 1-135
- Ryan, L.; Molyneuy, D.H.; Kuzoe, F.A.S.; Baldry, D.A.T. (1981). Traps to control and estimate populations of *Glossina* species. *Tropenmedizinische Parasitologie* 32: 145 - 148
- Saunders, D.S. (1962). Age determination for female tsetse flies and the age composition of samples of *G. pallidipes, G. palpalis fuscipes* and *G. brevipalpis*. *Bulletin of Entomological Research* 53: 579 - 595
- Symes, C.B.; Southby, R. (1938). The reduction of *G.palpalis* in a lake shore area by the „Block“ method. *Government Printer*, Nairobi
- Vale G.A., Hargrove J.W., Cockbill G.F. (1986). Field trials of baits to control populations of *Glossina morsitans morsitans* and *G. pallidipes* Austen (Diptera: Glossinidae). *Bull. of Entomological Research* 76, 179-193
- Vega, E.F.; Dow, F.P.; Bartelt, J.R. (1995). Dissemination of microbial agents using an autoinoculating device and several insect species as vector. *Biological Control* 5: 545 – 552
- Wall R., Langley P. (1991). From behaviour to control: the development of trap and target techniques for tsetse fly population management. *Agricultural Zoology Reviews* 4, 137-159

CHAPTER FIVE
GENERAL DISCUSSION AND RECOMMENDATIONS FOR FUTURE RESEARCH

It was shown in the field that male tsetse flies regularly contact other tsetse flies. The number of contact events was positively linear correlated with the apparent population density. With a few exceptions, these contacts occurred regardless of sex and age suggesting that the morphological differences between males and females were too small to be detected by the males. The results suggested further that male tsetse flies are attracted preliminary visually and that they may finally identify the sex and the willingness via contact.

The results further suggested that even other coexisting tsetse species of about the same size get regularly contacted.

However, there seemed to be preferences for the youngest and the oldest females and a discrimination against the youngest males. The reason for that was not clear being most probably due to either volatile chemicals or the fly behaviour. Yet, there may exist an effective olfactory repellent and attractant which deserves further investigations. A volatile attractant could attract the flies better to devices like traps or targets. A volatile repellent could prevent tsetse flies from sucking blood and therefore from transmitting the trypanosomes.

Strong evidence was obtained that females mate again when old. This would be a disadvantage for the 'Sterile Insect Technique' leading to a higher sterile : fertile male ratio required for this control method.

The youngest and oldest males did not contact other flies while males in wing-fray categories 2 - 5 were sexually most active.

Since contact events occur regularly between tsetse flies, the 'Dissemination Technique' has the potential to markedly increase the efficiency of a trap and reduce the number of traps necessary and hence, the costs for tsetse control. However, since contact events seem to be very rare at ultra low population density, no eradication may be achieved using this technique alone. Furthermore, the success of releasing reared contaminated males may be limited, because the youngest males seem to contact other tsetse flies only in rare cases. In case of coexisting tsetse species, only contaminated males of that species could be released which is easier to rear provided that the difference of their sizes cannot be detected from the males.

Fluorescent pigment powder can be used for further studies in detecting contact events between tsetse flies in the field. However, the time consuming marking procedure and the relatively low numbers of contact events suggest that a closed population on a small study site may be preferable for such studies. Field experiments should last as short as possible because the quality of marks decreased with time after initially marking the 'F0-generation' and the number

of contact events. Furthermore, it was shown that it is absolutely necessary to capture the flies individually.

This was the first study to investigate contact events between tsetse flies under real field conditions and to estimate the number of daily occurring natural contact events. However, more questions are to be answered regarding the potential of the 'Dissemination Technique' for the control of tsetse flies. For example, how efficient is an agent transmitted through contact? Do close range meetings also cause a transmission, if yes, to what extent? How many times can the same fly pass a CD in order to increase considerably the amount of picked agent and also the number and chance for successful transmission? Do tsetse flies recognise and avoid a trap once they have passed the device? For entomopathogenic fungi, it was shown there is a typical dosage-mortality response in the treatment of tsetse flies (Kaaya, 19899. Therefore, how many conidia are transmitted through contact events, and which germination rate would lead consequently to an infection? Do males of one tsetse species also contact tsetse flies of another species, if yes, to what extent? ...

The 'Dissemination Technique' must kill more flies than trapped or as in this case passing through the CD's so as to be considered as an alternative to trapping. Moreover, the 'Dissemination technique' may have to cause the morefold of mortality. This is in order to compensate various disadvantages. They include the delay of death after passing a CD, additional larvipositions of the infected females and additional costs through the mass production and

regular application of any agent. However, during the exposure of the contaminated CD's, less flies than those passing through the CD's were killed. That was because the infection rate of the CD was clearly less than 20 % and the number of infections through contact events were too low to compensate for that. Therefore, Maniania's CD contaminated with dry conidia of *M. anisopliae* would not be considered as an alternative to trapping.

Maniania's CD has to be modified in order to use better the positive phototaxis of tsetse flies. This will attract them faster into the CD and keep them there not for too long, so that all flies passing through the CD are still fit enough to transmit the conidia. This would mean that more sun light would be required to light up the CD. However, in this case, the conidia and therefore the germination rate may get more affected since they are vulnerable to sun light (Ferron, 1981). Yet, the germination rate may be improved due to a sticky formulation of the conidia. Chemical additives could be added in order to improve the conservation of the conidia (Ferron, 1981).

The use of biological agents for the 'Dissemination Technique' may be too inconvenient. This is because, in order to keep the biological agent as long as possible infectious, contamination of the used material must be prevented, and a refrigerator is necessary. Moreover, it seems necessary to produce the biological agent near to the field sites to avoid frequent long distance transportation. In contrast, long lasting and insensitive synthetic agents can be easier handled and longer kept favouring the central production which would

save the costs necessary for many production sites. In addition, they have to be reapplied not as often as biological agents. Moreover, if the synthetic agents are not vulnerable to sun light, the positive phototaxis of tsetse flies can be better used so as to bring all flies in a relatively short time through the CD. However, the existing synthetic agents are too expensive for the concerned African countries. Therefore, research should concentrate on cheap synthetic agents which are long lasting and insensitive to sunlight. This research should be conducted from the concerned countries in order to ensure that the agent is then cheaply available.

It seems not necessary to capture and keep the flies individually when investigating the infection rate of a tsetse population with *M. anisopliae*. Therefore, handling time and expenses could be reduced, when captured and kept together.

REFERENCES CITED

- Ferron, P. (1981). Pest control by the fungi *Beauveria* and *Metarhizium*. In „*Microbial control of pests and plant diseases 1970-1980*“ (H.D.Burges, Ed.), pp. 465-482. Academic Press, London.
- Kaaya, G.P. (1989). *Glossina morsitans morsitans*: Mortalities caused in adults by experimental infection with entomopathogenic fungi. *Acta Tropica* 46: 107 – 114

KENYATTA UNIVERSITY LIBRARY