

**Some aspects of the behaviour, ecology and
vectorial capacity of *Glossina brevipalpis*
Newstead and other tsetse flies in the
Luangwa Valley of Zambia**

M. VET. MED.
HIGGINS
M. A. S.
1999

by

Alikhadio Maseko

**Thesis submitted for the degree of Master of
Science in Veterinary Parasitology to the
Samora Machel School of Veterinary
Medicine, Department of Paraclinical Studies,
University of Zambia.**

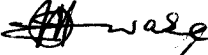
October 1999

APPROVAL

This dissertation of Alikhadio Maseko is approved as fulfilling the requirements of the award of the Degree of Master of Science in Veterinary Parasitology of the University of Zambia.

External Examiner

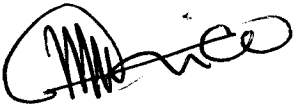
Supervisor 

Co Supervisor/Internal Examiner 

Chairperson, Board of Examiners 

Declaration

The contents of this thesis are the work of the author. The thesis has not been previously submitted for the award of a degree to any university.



Date 9/11/2000

Alikhadio Maseko

Acknowledgments

The work of this thesis was funded by The Regional Tsetse and Trypanosomosis Control Program (RTTCP) of Southern Africa and The Government of The Republic of Zambia through the Tsetse and Trypanosomosis Branch of the Department of Animal Production and Health. I wish to thank the two organisations for their financial, technical and material support.

I also wish to thank the RTTCP for according me the opportunity to carry out research at Kakumbi Field Research Station on a Research fellowship which culminated into the M.Sc. study.

I wish to thank the following people for their contribution towards making the research successful: Harding Chisanga (the late), John Silutongwe and Mutakela Kamungoma for helping in the collection of field data; to my field assistants, Meyam Njobvu, Godrick Mbao, Sangani Kumwenda and Masauso Mbewe for their hard work; the wildlife Police officers, Mike Zulu, Godfrey Muyunda and Pyela Nyirenda for ensuring our safety in the National Park.

I am grateful to Dr. L. M. Tuchili (Disease Control) who helped in the running of the PCR experiment in Chapter 6.

I am grateful to my supervisors Drs. H. Chitambo and E.T. Mwase-Ngulube, for not only reading through the thesis a countless times but

also for who endured more than 2000 km of rough roads to come and supervise my field experiments in the Luangwa Valley.

Lastly to Dr. Glyn Vale, my technical supervisor under RTTCP, I am most thankful for teaching me to be a scientist.

Dedication

To my wife, Astridah, for all the patience and tolerance during my absence from home.

To my children; Elly, Kambani and Chisomo for understanding in their own way that Dad is at School.

To my young brother Thomas and all my teachers to date; the only few who chose to be sources of knowledge.

Almighty God, do bless them all.

To my late father, David Sipikili Maseko, the true hero. Receive Gods everlasting peace.

TABLE OF CONTENTS

	Page Number
Title Page	i
Declaration	ii
Acknowledgements	iii
Dedication	v
Table of Contents	vi
List of Tables	x
List of Figures	xi
Abbreviations	xi
Abstract	xii
CHAPTER 1: Introduction and Literature Review	
1.1. General Introduction	1
1.2. Justification	3
1.3. Objectives	4
1.3.1. General Objectives	4
1.3.2. Specific Objectives	4
1.4. Literature Review	5
1.4.1. Tsetse Fly Distribution in Africa	5
1.4.2. Tsetse Fly Distribution in Zambia	8
1.4.3.1 Tsetse Fly Life Cycle	10
1.4.3.2 Tsetse Fly Host Location Behaviour	10
1.4.4. Distribution of Tsetse Flies Over Time and Space	13
1.4.5. Tsetse Fly Dispersal and Daily Displacement	14
1.4.6. Tsetse Fly Population Composition	15
1.4.7. Tsetse-Trypanosomosis Interface	16
1.5. <i>Glossina brevipalpis</i> Newstead	19
1.6. Trypanosomosis Control Methods	21
1.6.1. Chemotherapy	21
1.6.2. Trypanotolerance	22
1.6.3. Tsetse Fly Control Methods	22
1.6.3.1 Game or Host Elimination	23
1.6.3.2 Bush Clearing or Habitat Destruction	23
1.6.3.3 Sterile Insect Technique (SIT)	23
1.6.3.4 Control of Tsetse Flies by Use of Insecticides	24

1.6.3.4	Ground Spraying	24
.1		
1.6.3.4	Aerial Spraying	25
.2		
1.6.3.4	Cattle Dipping or Pour-On	25
.3		
1.6.3.4	Traps and Targets	25
.4		
1.7.	Study Area	27
1.7.1.	Location	27
1.7.2.	Climate	27
1.7.3.	Vegetation	27
1.7.4.	Land-use and other Economic Activities of the Luangwa Valley	28
1.8.	Structure of the Thesis	28

CHAPTER 2: Sampling Methods for *Glossina brevipalpis* Newstead and Other Tsetse Flies (Diptera: Glossinidae)

2.1.	Introduction	29
2.2.	Study Site	30
2.3.	Materials and Methods	31
2.3.1.	Experimental Design	31
2.3.2.	Fly Rounds	32
2.3.3.	Traps	33
2.3.4.	Traps and Electric Screens	33
2.3.5.	Fly Rounds with Electric nets	33
2.3.6.	Odours	34
2.3.7.	Refuges	34
2.3.7.1.	Preliminary Study	34
2.3.7.2.	Seasonal Use of Refuges	35
2.3.7.3.	Diurnal Use of Refuges	35
2.3.7.4.	Design of Refuges	35
2.3.7.5.	Refuges as Larviposition Sites	36
2.4.	Results	36
2.4.1.	Fly Rounds	36
2.4.2.	Traps	39
2.4.3.	Traps with Electric Nets	39
2.4.4.	Fly Rounds with Electric Nets	40
2.4.5.	Odours	41
2.4.6.	Refuges	42
2.4.6.1.	Preliminary Study	42
2.4.6.2.	Seasonal Use of Refuges	42
2.4.6.3.	Diurnal Use of Refuges	44

2.4.6.4.	Design of refuges	46
2.4.6.5.	Larval Deposition in Refuges	48
2.5.	Discussion	48

CHAPTER 3: The Distribution of *Glossina brevipalpis*, *G. m. morsitans* and *G. pallidipes* in Three Vegetation Types of the Luangwa Valley.

3.1.	Introduction	51
3.2.	Study Sites	53
3.3.	Materials and Methods	54
3.4.	Results	55
3.4.1	Traps	55
3.4.2.	Artificial Refuges	58
3.5.	Discussion	62

CHAPTER 4: Dispersal and Daily Displacement of *Glossina brevipalpis* in a Riverine Woodland of the Luangwa Valley.

4.1.	Introduction	67
4.2.	Study Sites	68
4.3.	Materials and Methods	69
4.4.	Results	70
4.5.	Discussion	73

CHAPTER 5: Composition of Catches of *G. brevipalpis*, *G. m. morsitans* and *G. pallidipes* from Epsilon traps and Artificial Refuges.

5.1.	Introduction	76
5.1.1.	Age Determination	79
5.1.2.	Wing Fray Analysis	80
5.1.3.	Ovarian Analysis	80
5.2.	Materials and Methods	81
5.2.1.	Fly Collection and Handling	81
5.2.2.	Dissection	82
5.3.	Results	83
5.3.1.	Epsilon Traps and Artificial Refuges	83
5.3.2.	Composition of Epsilon Trap Samples from Riverine, Mopane and Vegetation types	86
5.4.	Discussion	88

**CHAPTER 6: Species Specific Identification of Trypanosomes in
Glossina brevipalpis, *G. m. morsitans* and *G.
pallidipes* using Polymerase Chain Reaction (PCR)**

6.1.	Introduction	93
6.1.1.	<i>Duttonella</i> (<i>Vivax</i> Group)	94
6.1.2.	<i>Nannomonas</i> (<i>Congolense</i> Group)	95
6.1.3.	<i>Trypanozoon</i> (<i>Brucei</i> Group)	96
6.1.4.	Trypanosomes in Tsetse Flies	97
6.1.4.1.	<i>Duttonella</i> (<i>Vivax</i> Group)	98
6.1.4.2.	<i>Nannomonas</i> (<i>Congolense</i> Group)	98
6.1.4.3.	<i>Trypanozoon</i> (<i>Brucei</i> Group)	99
6.1.5.	Identification of Trypanosomes in the Tsetse Fly	99
6.1.6.	Distribution of Trypanosome Infection Rates in the Tsetse Fly	100
6.2.	Materials and Methods	101
6.2.1.	Collection of Infected Tsetse Flies	101
6.2.2.	Isolation and Purification of Trypanosome DNA	101
6.2.3.	Polymerase Chain Reaction (PCR) Amplification	102
6.3.	Results	104
6.3.1.	Development of the PCR Protocol	104
6.3.2.	PCR Amplification	104
6.4.	Discussion	106

CHAPTER 7: General Discussion 110

REFERENCES 115

ANNEXES

Annex 1	Tsetse Fly Species	131
Annex 2	Maps showing tsetse fly distribution in Africa, Zambia and working area	133
Annex 3	Figures of traps and artificial refuges	138
Annex 4	Tsetse fly marking positions and scheme	143
Annex 5	Wing fray and age estimation	146
Annex 6	Tsetse fly dissection and ageing of females	149
Annex 7	PCR protocol	151

List of Tables

2.1	Catches using epsilon traps, MFR & BFR	38
2.2	Catches using epsilon traps with and without electric screens	40
2.3	Capture efficiency of fly rounds for tsetse flies attracted	40
2.4	Catches of tsetse flies in various modified refuges	47
3.1	Summary of annual catches of tsetse flies from traps	57
3.2	Species ratio in different vegetation types	57
3.3	Average epsilon trap catches in each vegetation type	58
3.4	Summary of tsetse fly catches from artificial refuges	59
4.1	Summary of the mark recapture experiment for <i>G. brevipalpis</i>	72
5.1	Results from dissection data from artificial refuges and traps	83
5.2	Age distribution of female tsetse flies from epsilon and artificial refuges	85
5.3	Age distribution of male tsetse flies from epsilon and artificial refuges	85
5.4	Results of tsetse fly dissections in riverine, mopane and thicket vegetation	86
5.5	Age distribution of female tsetse flies from refuges in riverine, mopane and thicket vegetation types	87
5.6	Age distribution of male tsetse flies from refuges in riverine, mopane and thicket vegetation types	88
6.1	Results of the PCR amplification	105

List of Figures

I	Seasonal refuge catch for <i>G. brevipalpis</i>	43
II	Diurnal refuge pattern	45
III	The seasonal trend of tsetse fly catches in the riverine vegetation	61

List of Abbreviations

1	BFR	Black screen Fly Round
2	DRC	Democratic Republic of Congo
3	ELISA	Enzyme Linked Immuno Sorbent Assay
4	FAO	Food and Agriculture Organisation
5	GMA	Game Management Area
6	GRZ	Government of the Republic of Zambia
7	ILRAD	International Laboratory for Research on Animal Diseases
8	ISCTRC	International Scientific Council on Trypanosomosis Research and Control
9	MFR	Man Fly Round
10	MWV	Mean Wing Fray Value
11	OAU	Organisation of African Unit
12	PBS	Phosphate Buffered Saline
13	PCR	Polymerase Chain Reaction
14	RTTCP	Regional Tsetse and Trypanosomosis Control Program
15	SEMG	Special Environmental Monitoring Group
16	SIT	Sterile Insect Technique
17	SLAMU	South Luangwa Area Management Unit
18	UNZA	University of Zambia
19	UV	Ultra Violet Light

ABSTRACT

Studies were carried out in the South Luangwa National Park, located in the Luangwa Valley Zambia, on the behaviour, ecology and vectorial capacity of *G. brevipalpis* and *G. m. morsitans* and *G. pallidipes*. The catch of *G. brevipalpis* was low in odour baited epsilon, NGU2G, F3 and biconical traps throughout the year. It was, however, observed that the flies were attracted to the traps but did not enter. *Glossina brevipalpis* was not easy to catch with ordinary fly rounds, but with the use of an electric screen fly round, more of this fly were caught. The best sampling system for *G. brevipalpis* was found to be artificial refuges which worked better in the hot dry season from mid-August to mid-December. Tsetse flies started entering refuges from 09:00 h and left after 16:00 h and this is the time in which tsetse flies rest because the environmental conditions negate tsetse fly survival during this period. Refuges with a total volume of at least 200 liters were more efficient for as long as they were dark, insulated and provided with shade. *Glossina m. morsitans* was caught more on fly rounds than using traps while, *G. pallidipes* was captured more with traps than in any other sampling system used. Like *G. brevipalpis*, both *G. m. morsitans* and *G. pallidipes* responded well to artificial refuges.

Glossina brevipalpis was concentrated in the riverine vegetation type throughout the year while *G. m. morsitans* and *G. pallidipes* were more concentrated in the mopane and thicket vegetations. *Glossina brevipalpis* was also observed to be a very localised fly to the riverine vegetation and rarely leaves it. Using a circular setting of seven artificial refuges of 300 m radius to the one centrally placed gave a recapture rate of 7% over a period exceeding three weeks. It was noted that even in such a small area used some refuges outside the riverine vegetation but within the said radius did not capture any *G. brevipalpis*.

Although the catches for artificial refuges and traps were different in terms of species composition, the age distribution by both ovarian and wing fray analysis were similar. There was also no significant differences in infection rates of *G. brevipalpis* (3.3%), *G. pallidipes* (3.5%) and *G. m. morsitans* (4.4%).

Species identification of trypanosomes were investigated for *G. brevipalpis* using a protocol which was developed specifically to isolate trypanosome DNA in tsetse fly parts for amplification by the PCR technique. The technique was able to identify 100% of the *T. congolense* (Savannah) and 43% of the *T. congolense*-like infections in tsetse fly midguts were identified to be *T. brucei*. *Trypanosoma vivax* and *T. simiae* primers did not amplify and reasons for this are suggested.

Glossina brevipalpis was therefore, shown to be a potential vector of African trypanosomosis and hence further studies on the ecology and control of this species are recommended.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1. GENERAL INTRODUCTION

Tsetse flies and the trypanosomes which they carry as biological vectors are partners in a 'widespread crime' (Nash, 1969). The resulting disease called trypanosomosis affects both man and livestock. The disease in man is called sleeping sickness, nagana in cattle, sheep and goats while it is called surra in camels. Trypanosomosis is one of the most economically important diseases of livestock in Zambia and about a third (250,000 km²) of the total arable land is tsetse infested (Mumba & Chizyuka, 1987). It is also reported that over 60% of the cattle population in Zambia is at risk to the disease (Akafekwa & Awan, 1975).

Tsetse flies infest approximately ten million square kilometers of Africa covering 38 countries (Murray & Gray, 1984). It is considered that seven million square kilometers of this area would otherwise be suitable for livestock and crop production (FAO, 1980). About 30% of the 147 million cattle in the countries affected by tsetse flies are exposed to the disease (ILRAD, 1993). Trypanosomosis in cattle is thus one of the most important constraints to rural development in Africa (Murray & Gray, 1984). The trypanosomosis situation in sheep, goats, pigs, horses, donkeys and camels is probably similar but this is not very well

documented (ILRAD, 1993). The estimated losses due to trypanosomosis is different in different places, however, the annual losses in livestock production due to African trypanosomoses were in 1983 estimated at five billion United States Dollars (Murray & Gray, 1984), and no reports to date indicate a different scenario. It has also been estimated that the total cost of controlling the disease in 1992 alone was in excess of US \$500 million (ILRAD, 1993). However, present data seem to indicate that tsetse flies are on the contrary spreading to new areas in Zambia (GRZ, 1996). Since the 1950s, the area infested by the Savannah tsetse fly species has thus continued to expand except in a few areas where due to high human population densities tsetse fly fronts have declined (Jordan, 1979). This trend has consequently increased the pressure for settlement in tsetse fly free areas (Murray & Gray, 1984).

There are 30 different species of tsetse flies found in Africa today. They belong to three distinct groups of *Glossina* namely the *Morsitans*, *Palpalis* and *Fusca* which are grouped according to their similarities mainly in terms of type of habitat and behaviour (see annex 1). Five species of tsetse flies have since been reported in Zambia and they are *Glossina morsitans morsitans* Westwood, *G. morsitans centralis* Muchado, *G. pallidipes* Austen, *G. brevipalpis* Newstead, and *G. fuscipes fuscipes* Newstead (GRZ, 1996).

In order to control trypanosomosis and their tsetse flies vectors, more effectively, the international initiative has undertaken several measures which include research in tsetse flies trapping and control, development of better trypanosome diagnostic techniques and training of relevant personnel (ILRAD, 1993). However, research on tsetse flies has advanced only for certain species of tsetse flies especially the *Morsitans* group, while work on the *Palpalis* and *Fusca* groups has lagged behind (Vale, 1993).

1.2. Justification

While the resource position of most countries which are tsetse fly infested has declined, the tsetse problem has continued to grow. In order to rationalize the use of the scarce resources, priorities in tsetse fly and trypanosomosis control need to be addressed. These priorities should be based, among other things, on proper understanding of the ecology of the tsetse vectors in the areas of concern. This, therefore, means an acquisition of adequate knowledge on the vector in terms of its behaviour and population dynamics, which include population composition, mobility and distribution over time and space, preferred hosts and species of trypanosomes they carry, becomes very important. The vectorial capacity of *G. morsitans morsitans*, *G. morsitans centralis* and *G. pallidipes*, is well documented in Zambia (Williemse, 1991; Woolhouse *et al.*, 1993, Bealby *et al.*, 1996). However, the role of *G. brevipalpis* and *G.*

fuscipes fuscipes in the epidemiology of trypanosomosis in Zambia is not well known. It was with this background that this research was carried out in order to understand some aspects of the behaviour, ecology and vectorial capacity of *G. brevipalpis* in relation to *G. m. morsitans* and *G. pallidipes*. Although *G. brevipalpis* is generally considered to be of no economic importance, it was reported to have caused an enormous trypanosomosis disease problem in the Zulu land of the Republic of South Africa (Kappmeire, 1997).

1.3. Objectives

1.3.1. General Objective

To study the behaviour, ecology and vectorial capacity of *Glossina brevipalpis* and other tsetse fly species of the Luangwa Valley, Zambia.

1.3.2. Specific Objectives

1. To study the behaviour and the response of *G. brevipalpis* to different sampling systems.
2. To investigate the distribution of *G. brevipalpis* over time and space.
3. To elucidate the dispersal and daily displacement of *G. brevipalpis*.
4. To study population composition of *G. brevipalpis* in terms of physiological age and trypanosome infection rates.

5. To identify the type of trypanosomes present in the tsetse fly mouthparts, mid-gut and salivary glands using Polymerase Chain Reaction (PCR).

1.4. LITERATURE REVIEW

1.4.1 Tsetse Fly Distribution in Africa

The genus *Glossina* is restricted only to Africa (Buxton, 1955). Its northern limits (14° North) extends across the continent from Senegal in the west to southern Somalia in the east which corresponds closely to the southern limits of the Sahara and Somali Deserts. The southern limits are however, less well defined. In the southwest, it varies between 10° and 20° South, corresponding closely to the northern edges of the Kalahari and Namibian Deserts, where as in the South-east it is generally at about 20° South but extends as far as 29° South along the east African littoral (Jordan, 1986). These limits are determined by climate, often through its effect on vegetation (Jordan, 1986; FAO, 1992). Where the limits are near to the margins of deserts, the 500 mm isohyet is close to the limits although the flies can penetrate drier areas within the shelter of vegetation lining water courses (Bursell, 1960). In southern Africa, where the rainfall is high and there are no deserts, the limits of *Glossina* is related to seasonal low temperatures (Jordan, 1986). This may affect the adult tsetse flies, which are inactive at temperatures below 16°C. The low temperatures also prolong the period spent in the soil as

puparia, this leads to all the preserved fat in the pupae being used before the development into the adult stage can be completed (Bursell, 1960). With these natural limitations in the development, infestations of *Glossina* are not continuous (Ford & Katondo, 1977). Extensive areas devoid of trees, whether naturally occurring or as a result of human activities, are generally free of tsetse flies. This is also observed in areas of high altitude (Swynnerton, 1936; Nash, 1969; Jordan, 1986). The altitudinal limitation on tsetse fly distribution is also related to seasonal low temperatures though it tends to be rather difficult to define (Jordan, 1986; Nash, 1969). The general distributions of the three tsetse fly groups are described by the FAO (1980). A complete list of the tsetse fly species according to their grouping is presented in Annex 1.

In general, the *Morsitans* group of tsetse flies do not live in very wet areas such as the rainy forest and mangrove swamps but are found mainly in the open Savannah areas of Africa. Their distribution seems to be limited by cold winter conditions in the south (*G. m. morsitans*, *G. m. centralis*, *G. pallidipes* and *G. austeni*) [Zimbabwe, Botswana], and hot dry conditions in the north of West and Central Africa (*G. m. centralis* and *G. pallidipes*) (see Map 1 in Annex 2). In less severe climatic conditions, their distribution may be limited by scarcity of animals such as game animals on which the tsetse flies feed and to some extent, due to the lack of trees which provide the necessary shade (FAO, 1980).

The *Palpalis* group of tsetse flies are mainly restricted to the very humid areas of Africa which include the mangrove swamps, the rain forest, the lake shores and the gallery forests along rivers. When members of this group, penetrate the drier areas, they tend not to move far from the free water bodies like the rivers and lakes although in more humid areas, the *Palpalis* tsetse flies may not have to necessarily live close to free water bodies (See Map 2 in Annex 2) (FAO, 1980). *Glossina palpalis* lives in the more humid areas of West Africa, from Senegal to Cameroon then along the coast to Angola. In West Africa it penetrates further to the north in Mali and Senegal, than it does in Nigeria. In the part of its distribution from Cameroon to Angola, it has a long common boundary with *G. fuscipes*, with which there is little overlap. *Glossina fuscipes* occupies a very large inland block of Africa centered on the Democratic Republic of Congo, but covering some of the land in all of the countries surrounding the Democratic Republic of Congo, as well as Gabon, Cameroon and the southern part of Chad. *Glossina tachnoides* is distributed in a broad belt from Guinea in the west, to the Central African Republic in the east. In addition, much further to the east are some smaller isolated belts on the Sudan - Ethiopia border. In Nigeria and Chad this species is able to live further north than either *G. palpalis* or *G. fuscipes*. *Glossina calliginea* and *G. pallicera* are mainly limited to mangrove and thick forest of west Africa, as far as Gabon.

The *Fusca* group of tsetse flies are of three types mainly on the basis of distribution. *Glossina longipennis* is confined to Sudan (south east corner), Ethiopia (southern boarder), Somalia, Kenya and Tanzania (north eastern quarter). In general, *G. longipennis* mainly inhabits the dry areas of the land. *Glossina brevipalpis* is widely distributed in the eastern part of Africa, stretching from Ethiopia and Somalia in the north, to Mozambique and South Africa in the south. There is a large belt of *G. brevipalpis* in an area west of Lake Tanganyika in the Democratic Republic of Congo. The other well known pockets of the *Fusca* group of tsetse flies are mainly restricted to the thicker rain forest areas of Africa. They have a distribution pattern similar to that observed for the *Palpalis* species especially *G. palpalis* and *G. fuscipes*. In contrast, the *Fusca* group of tsetse flies do not usually penetrate very far into the gallery forest (See Map 3 in Annex 2) and thus, its distribution is more limited than the *Morsitans* and *Palpalis* groups (Ford & Katondo, 1977).

1.4.2. Tsetse Fly Distribution in Zambia.

According to the National Strategic Plan for tsetse fly and trypanosomosis in Zambia, three tsetse fly belts are recognised (See Map 4 in Annex 2).

(a) The Eastern Fly Belt: It Comprises approximately 170,000 km² of land stretching in a north-eastern direction beginning from the confluence of the Kafue and Zambezi Rivers in Lusaka Province and from the Luangwa

River Valley to its approximate northern limits near the northern boundary of the country (GRZ, 1996). Wet season intrusion of tsetse flies occurs on the fringes of the tsetse fly belt, especially in the high agricultural potential area of eastern Kabwe. The Game Management Areas (GMAs) and national parks (North and South Luangwa, Lukusuzi and Nyika) constitute a major portion of the fly belt. The fly species found in the belt are mainly *G. morsitans morsitans*, *G. pallidipes* and *G. brevipalpis*.

(b) The Western Fly Belt: This belt Stretches from the south-western corner of the country through Senanga and Sesheke districts of Western Province in a north-easterly direction of the Zambezi River and then northwards through Kaoma towards the Zambia-Democratic Republic of Congo border. The Kafue and Siomagwezi National parks and its GMAs are encompassed within its boundaries. The southern limit of the belt still remains undefined. The tsetse fly species found in this belt are *G. morsitans centralis* and *G. pallidipes*.

(c) Luapula/Northern Tsetse Fly Belt: There are other smaller pockets of tsetse fly belts in Luapula Province around Lakes Bangweulu, Mweru and Tanganyika. The tsetse fly species found in these areas are *G. fuscipes fuscipes* and *G. brevipalpis*. Some *G. m. centralis* and *G. pallidipes* have also been recorded. Because these areas are marginal livestock production areas, they are not considered to be economically

important foci for animal trypanosomosis and cases of human trypanosomosis (sleeping sickness) are also very rare.

1.4.3.1 Tsetse Fly Life Cycle

Tsetse flies, both male and female are hematophagous insects which solely depend on host blood for survival (Buxton, 1955). Tsetse flies exhibit a unique life cycle called adenotrophic viviparity where one egg is ovulated, fertilized and nourished by the female tsetse fly until it is larviposited (FAO, 1980). The larva quickly burrows into the soil and the pupa stage has begun. The pupa stage lasts about 3-5 weeks depending on the season with the pupal stage being shortest in the hot dry season and longest in the cool dry season (*Ibid*). Female tsetse flies "lays" larva every 9-10 days of their lives except for the first pupa which is dropped 18 days after hatching of the female tsetse fly. The female mates only once in its life time and the sperm from the male is kept in the spermatheca in the female and it remains viable for the whole of the female flies' lifetime (*Ibid*).

1.4.3.2 Tsetse Fly Host Location Behaviour

Information on insect behaviour is needed in order to improve baits currently being used for surveying and for controlling insect populations (Vale, 1993). The most important aspect of tsetse fly behaviour relates to host location. It is the host location series of stimuli that are used to

attract and trap tsetse flies in traps, fly rounds (Man, black screen and ox), and targets (Vale, 1974a; Flint, 1985). In trypanosomosis epidemiology, it is also the tsetse fly feeding behaviour which eventually leads to sucking of blood, an act which may result in effecting transmission of trypanosomes and the subsequent infection of the host to cause trypanosomosis .

When tsetse flies are looking for a host, they use various sensory perceptions (FAO, 1992). For a long time, it was thought that visual perception was the most important mode of perception involving the discernment of colour, contrast with the environment, and movement (Green, 1994). The fore-mentioned concept was despite the fact that early work had indicated that odour was another important factor in the ability of the tsetse flies in locating their mammalian hosts (Persoons, 1966; Langridge, 1960; Hughes, 1957 a & b; Vanderplank, 1944; Bax, 1937, Lloyd, 1935; Chorley, 1933). It was not until recently that the use of certain odours was developed and utilised for tsetse fly control and research purposes (Vale, 1974a, 1993 & 1998).

A significant advancement in the use of baits came with the introduction of electric nets for sampling tsetse flies in the absence of man (Vale, 1974a). These experimental designs allowed an assessment of the extent of olfactory attractions of tsetse flies to odours. It is now known that the

odour from hosts can act on the flies in several ways: 1) Carbon dioxide stimulates tsetse to fly and start searching for the host; 2) acetone and octenol induces tsetse fly flight towards the host; 3) 3-n-propyl phenol and 4-methyl phenol promote entry of tsetse flies into traps (FAO, 1992). Tsetse flies generally fly upwind towards the odour source when they detect an attractive odour (Vale & Hargrove, 1979; Brady & Griffiths, 1993; Groenendijk, 1996). When they lose the scent in flight, they turn sharply, and may settle and re-assess the wind direction before flying upwind again (Groenendijk, 1996). Tsetse flies can actually assess the wind direction whilst in flight (Brady & Griffiths, 1993). When the tsetse flies get close to the host, they then begin to use their sight to make the final approach for landing (FAO, 1992). However, flying upwind to find the odour source may be difficult under field conditions, especially if the vegetation is dense, and where the wind is constantly changing directions, under such circumstances the tsetse fly may even lose its direction (Brady & Griffiths, 1993). Nevertheless, it is now thought that even if the odour can divert the tsetse flight, it still gives the fly a good chance of finding the host (Groenendijk, 1996). There is also added evidence that the tsetse fly may reduce their flight speed and turn more frequently when they detect high odour concentrations (Brady & Griffiths, 1993). It is reported that the range of attraction for the ox odour is about 90 m down wind, but it may be less for the synthetic baits (Brady & Griffiths, 1993; FAO, 1992). The range of visual attraction of a

stationary trap is estimated to be between 15 and 20 meters, but it is more than that for a moving object (Green, 1986; Green & Flint, 1986; Flint, 1985). It is also now known that Pthalogen blue (Royal Blue) and white colours are more attractive from a distance, while black is good for landing responses (Green & Flint, 1986). Commonly used odour baits are carbon dioxide, acetone, butanone, 1-octen-3-ol, 3-n-propyl phenol and 4-methyl phenol (Vale & Hall, 1985; Vale *et al.*, 1988ab; Torr, 1989). The artificial baits which use coloured cloth and artificial odours are the pillars of the environmentally more friendly target and trap technology for tsetse fly control in the field today (Vale *et al.*, 1985; Vale, 1998).

1.4.4. Distribution of Tsetse Flies Over Time and Space

The nature of certain factors which limit the distribution of the various species of *Glossina* is still unknown. However, it is known that the vegetation, which is locally influenced by the climate and to some extent the soil, affects the distribution of tsetse flies (Jordan, 1986). The presence of a suitable food source is also essential for the survival of the tsetse flies and therefore, it has an influence on the distribution of tsetse flies. Because stress is greatest near the limits of the distribution of most plant and animal species, it follows that any type of activity directed towards controlling any species is most likely to be successful in marginal habitat conditions and that the chance of success decline as you approach the optimal habitat conditions (Jordan, 1986). Attempts to

control or to locally eradicate any species basically adds a certain degree of stress which is already presented to that species by the local environment (Vale *et al*, 1986, 1988a).

Understanding of the relationship between the vegetation types and the seasonal tsetse fly density is very crucial in our attempt to control tsetse fly populations, and subsequently to help break the trypanosome transmission cycle. Good knowledge on tsetse fly ecology, can help to identify the vital intervention points which can be exploited to induce certain stress factors that can lead to decline in tsetse fly populations (Hargrove, 1981b; Glasgow, 1963).

1.4.5. Tsetse Fly Dispersal and Daily Displacement

Jackson (1941, 1949) suggested, on the basis of mark-recapture experiments, that male *Glossina morsitans morsitans* Westwood were generally restricted in their movements to "ambits" (restricted habitat) whose limits they rarely exceeded. Bursell (1970) however, argued that Jackson's data and later information on tsetse flies were better explained on the basis of a model of restricted movement. Rogers (1977) developed the model quantitatively and extended it to cover other tsetse fly species. Random movement is sufficient to explain most models of tsetse movement although in reality, tsetse fly movement is more complex (Hargrove, 1981a). Mark-recapture studies indicate that there are

important age-dependent changes in tsetse fly availability as observed using almost all known sampling techniques (Hargrove, 1981a). While complex models are important in understanding vector ecology, simple measurement on how far tsetse flies move within their habitat and their turn over rate in a given population within a specified habitat, is useful information for efficient tsetse fly control and assessing its vectorial potential for trypanosomes.

1.4.6. Tsetse Fly Population Composition

Populations of animals are affected by both density dependent and density independent mortalities (Rogers, 1979; Hargrove, 1991). Whilst the average of the sum total of both sorts of mortality determine mean population size, only density dependent mortalities regulate abundance about that mean level. Tsetse fly mortality rate is therefore, higher than average when populations are higher than average and *vice versa* (Rogers, 1979). The obvious effects of weather on tsetse fly survival demonstrates the importance of abiotic mortalities. The effects of temperature and saturation deficit are not clearly linked to the tsetse fly abundance and distribution while light intensity has a considerable effect on its abundance as observed by Harley (1965).

These factors have an effect on the longevity of tsetse flies such that female tsetse flies survive twice longer than male tsetse flies hence the

natural population of tsetse flies has twice as many females as males (FAO, 1992). It is observed that 67% of the tsetse population is comprised of females flies while the rest are males. In studying population composition of tsetse flies, it becomes important to explain sample composition in light of the expected ratios of males and females in natural populations (Hargrove, 1991).

1.4.7 Tsetse-Trypanosomosis Interface

The disease trypanosomosis is caused by various species of *Trypanosoma*, a genus of parasitic protozoa found in the blood and tissues of their hosts. There are many species of *Trypanosoma* found in a wide range of vertebrate species throughout the world, some of them causing disease while some tend to be harmless (Urquhart *et al* 1996; Jordan, 1986; Soulsby, 1986). Trypanosomes are usually transmitted from one vertebrate host to another by hematophagous arthropods, although leeches are also vectors of trypanosomes of fish and amphibians. In Central and South America the vampire bat, *Desmodus rotundus*, has been incriminated in the transmission of *Trypanosoma evansi* to man and other mammalian hosts (Soulsby, 1986). *Trypanosoma equiperdum* is sexually transmitted through coitus in donkeys and horses. This species is exceptional in that no intermediate host is involved (Soulsby, 1986). Transplacental transfusion, salivaria, stercolaria and blood transfusion can also be modes of transmission of

trypanosomosis. The stercolaria mode of transmission is of major importance in the epidemiology of *T. cruzi* which causes Chagas disease in South America. The stercolaria transmission occurs through contact with infective faeces of the arthropod vector, the reduviid bugs (Soulsby, 1986; Urquhart, *et al.*, 1996). It is also known that transmission can be effected by carnivores through eating fresh raw trypanosome infected prey meat (Jordan, 1986; Mulligan, 1970; Ormerod, 1967; Hoare, 1972).

In Africa, most species of *Trypanosoma* are biologically transmitted from one vertebrate host to another mainly by tsetse flies (Boyt, 1980). The normal vertebrate host of these trypanosomes are the large wild mammals of Africa which, unless stressed, generally do not suffer any pathogenic effects of the trypanosomes (Mulla & Rickman, 1988). Some of these animals can harbour trypanosome infections for very long periods of time, perhaps indefinitely, and generally act as reservoir hosts from which tsetse flies can acquire infections (Mulla & Rickman, 1988). Once infected, tsetse flies themselves become reservoirs of the parasites as trypanosomes undergo cycles of development within the fly. Any fly with the infective forms of trypanosomes (metacyclics) has the potential to infect any animal from which it will subsequently take a blood meal. This transmission of trypanosomes between wild animals and tsetse flies is of no practical economic significance until man or his domestic animals, which are usually susceptible to the pathogenic effects of

trypanosomes, intrude into the cycle and become hosts of the parasites (Jordan, 1986; Mulligan, 1970; Nash, 1969; Buxton, 1955). Sleeping sickness in man and nagana or surra in domestic animals which are as a result of trypanosome infections, can be fatal unless treated or revert to chronic manifestation which is a cause of insidious production losses.

All those *Trypanosoma* species which undergo a cycle of development in the tsetse fly belong to the *Salivaria* group of the family Trypanosomatidae and in the order Kinetoplastida (Soulsby, 1986). There are four subgenera of salivarian trypanosomes namely *Duttonella*, *Nannomonas*, *Trypanozoon* and *Pcynomonas*. The subgenus *Duttonella* contains *T. vivax* and *T. uniforme* and they are both reported to develop in the proboscis of tsetse flies (FAO, 1980). The subgenus *Nannomonas* is composed of *T. congolense* and *T. simiae* which are said to develop in the proboscis and midgut (FAO, 1980). The subgenus *Trypanozoon* has three sub-species, *T. brucei brucei*, *T. b. gambiense* and *T. b. rhodesiense* (FAO, 1980). The *Trypanozoon* subgenus is said to develop in the proboscis, midgut and salivary glands (FAO, 1980). The subgenus *Pcynomonas* which contains only *T. suis*, is of limited distribution and importance (Jordan, 1986). It develops in the mid-gut, proboscis and salivary glands (FAO, 1980).

It is generally documented that the most common trypanosome in livestock is *T. congolense* followed by *T. vivax* while *T. brucei* is rare (Lubinga, 1996). However, it is reported that the most common trypanosome in tsetse flies, is *T. vivax* followed by *T. congolense* while *T. brucei* is again rare (Bealby *et al.*, 1996). Since the major source of infection in mammals are tsetse flies, then the tsetse fly and cattle infections require more investigation. The use of different diagnostic techniques which employed parasitological, Polymerase Chain Reaction (PCR), antigen-ELISA and Woo method on given samples of bovine blood from cattle in Petauke district of Zambia, revealed infection ratio of *T. congolense*: *T. vivax*: *T. brucei* of approximately 1:1:1 in cattle (Lubinga, 1996). It will be interesting therefore, to investigate the infection rates in different tsetse flies using more sensitive techniques in order to establish the actual infection types and their distribution in the tsetse fly proboscis, midgut and/or salivary glands.

1.5. *Glossina brevipalpis* Newstead

Increasing land pressure in the tsetse fly infested areas is leading to more utilization of the forest lands for livestock grazing (Jordan, 1979). This inevitably promotes the contact between livestock and forest dwelling tsetse flies such as *Glossina brevipalpis* (Kiragu *et al.*, 1997). The importance of *G. brevipalpis* in the epidemiology of African trypanosomosis has been ignored as it is reflected in the limited

literature (FAO, 1992; Nash, 1969; Bealby *et al.*, 1996). The out breaks of trypanosomosis in the Zululand of the Republic of South Africa and elsewhere especially in Kibwezi, Kenya, where *G. brevipalpis* is implicated has brought about a revived interest to an old problem of man and livestock coming in contact with tsetse fly populations (Kiragu *et al.*, 1997; Kappmeirre, 1997). In natural populations of *G. brevipalpis*, trypanosome infection rates of up to 3.5 % have been recorded (Harley, 1967; Kiragu *et al.*, 1997).

Glossina brevipalpis is a crepuscular tsetse fly; it is most active very early in the morning and very late in the afternoon (FAO, 1980). It is also the only tsetse fly that has a 24 hours diel activity (Harley, 1965). *Glossina brevipalpis* has been observed to sit and wait along animal routes unlike the other tsetse flies which follow the animal hosts (Harley, 1965; Swynnerton, 1936). In flight, it produces a distinct drowning sound which is peculiar only to this tsetse fly species (FAO, 1980).

Recently, efforts have been stepped up to try and understand the ecology of *G. brevipalpis* as a vector of animal trypanosomes. The work of Kiragu *et al.*, (1997) and Kappmeirre (1997) have highlighted the importance of *G. brevipalpis* as a vector of trypanosomosis in areas where this species of tsetse flies has come into contact with man and livestock. In this study

efforts have been made to further highlight the ecology of *G. brevipalpis* and its trypanosome vectorial capacity.

1.6. Trypanosomosis Control Methods.

Trypanosomosis can be controlled by both direct and indirect methods. The direct methods involve a direct attack on the trypanosomes while the indirect methods involve control of the tsetse fly vector and the maintenance of trypanotolerant livestock (Jordan, 1979). The above trypanosomosis control methods are further discussed below.

1.6.1. Chemotherapy

Trypanocidal drugs are in wide spread use in Africa both in traditional livestock systems and under ranching conditions (Williams *et al.*, 1993). In both situations, regularization of drug regimes would result in reduction of the risk of development of resistance to the relatively limited range of drugs (Boyt, 1980). Drugs do not present the final answer to the trypanosomosis problem, but their judicious use can reduce the disease to manageable levels which can allow land development, including the destruction of the tsetse fly habitat, to take place (Jordan, 1979 & 1987). In both East and West Africa, beef production can be maintained under drug protection even under high tsetse fly challenge (Bylis *et al.*, 1994; Williams *et al.*, 1993). The key is good nutrition and close attention to animal health and drug regimes. Because of the ensuring such

conditions that is good nutrition and good veterinary attention, however, ranches are often not economically viable (Williams *et al.*, 1993). The strategic use of drugs, under good veterinary supervision and management, can similarly enable oxen to survive and work in areas of high trypanosomosis challenge. In areas of low tsetse fly challenge, there is much evidence, that even the supposedly trypanosomosis susceptible breeds like Zebu can tolerate the local strains of trypanosomes, if initially they are protected by drugs (Williams *et al.*, 1993).

1.6.2. Trypanotolerance

Many wild hosts of trypanosomes are innately resistant to the pathogenic effects of infection (Mulla & Rickman, 1988). Among cattle, *Bos taurus* breeds are more tolerant than *Bos indicus*, while European breeds show no resistance at all (Williams *et al.*, 1993). Trypanotolerant cattle such as Mtoro and N'dama could be more widely used, but the *Bos taurus* only forms 5% of the estimated 147 million cattle in areas affected by tsetse flies (ILRAD, 1993). At present breeding rates, it would take 15 years to double the N'dama. Perhaps we should first ask why, after 7,000 years N'dama are still so rare among cattle herds of Africa?

1.6.3. Tsetse Fly Control Methods

Several methods of tsetse fly control have been used in the past and at present, some of these are discussed below.

1.6.3.1. Game or Host Elimination

The earliest methods used to control tsetse flies involved eliminating game animals (wild hosts of tsetse flies) (Buxton, 1955). The rinderpest panzootic of 1895 was followed by the disappearance of tsetse flies from the Limpopo and Zambezi basins except for a few, small, widely separated foci showing that the elimination of wild hosts would effectively eliminate tsetse flies (Vale & Cumming, 1976). This method was applied judiciously with some success but also to the detriment of the wild life resource. Tsetse flies also changed their host preferences to the only surviving species of mammals, birds and reptiles (Vale & Cumming, 1976).

1.6.3.2. Bush Clearing or Habitat Destruction

Tsetse fly habitat is removed by making the vegetation more open (Buxton, 1955; Nash, 1969). This method also involves the destruction of valuable resources. In fact bush clearing may even lead to soil erosion due to the removed vegetation cover and the heavy machinery used to clear the land (Jordan, 1986).

1.6.3.3. Sterile Insect Techniques (SIT)

Tsetse flies are reared in laboratories and radiation is used to sterilise males which are then released (Curtis & Langley, 1972; Jordan & Curtis,

1972). Since females only mate once, this effectively produces sterility in the females that mate with sterilised males. Although the technique has been shown to work, it is only economically viable if complete eradication can be assured (Williamson *et al.*, 1983). It also leads to an increase in the incidence of trypanosomosis in the short run by increasing the population of tsetse flies hence increasing the density and activity of the vectors of the disease which translates into more disease. (Jordan, 1986).

1.6.3.4. Control of Tsetse Flies by Use of Insecticides

The control of tsetse flies using insecticides started in the late 1940s after the discovery of DDT (Du Toit, 1954). Ever since that time, control of trypanosomosis has relied heavily on the control of the tsetse fly vector by the use of insecticides using several techniques. The commonly used insecticides in Zambia today are synthetic pyrethroids especially Delta methyrin and α -cyper methrin.

1.6.3.4.1. Ground Spraying

This method involves the spraying of insecticide to the tsetse fly habitat. Endosulphan and DDT have been used successfully to control tsetse flies in many parts of Africa (Du Toit, 1954; Jordan, 1986). This method is no longer used due to the deleterious effects the chemicals have on the *enviroment which include the following; large scale contamination of the*

environment and the accumulation of the chemical in the high order carnivores and birds of prey, because the chemicals are not biodegradable (SEMG, 1993).

1.6.3.4.2. Aerial Spraying

This method involves the spraying of insecticide to the tsetse fly habitat from the air in small droplets (30 μm) (Allsop & Coutts, 1977). Fixed wing aeroplanes and helicopters are used. Several sprays are made to the tsetse fly habitat up to five times and the interval of spray is dependent on the inter-larval period (FAO, 1980). It is a very effective method of tsetse control, however, it is very expensive.

1.6.3.4.3. Cattle Dipping or Pour-On

Cattle can be used as live 'targets' with insecticides applied on them either by dipping, pour-on or spraying (Dransfield *et al.*, 1991). Where a dipping infrastructure exists, this method may be cheap, but to effectively reduce tsetse fly densities, the cattle population densities must be reasonably high and fairly evenly distributed in the tsetse fly habitat (Dransfield *et al.*, 1991).

1.6.6.4.4. Traps and Targets

Our inability to sustain long-term control in most areas using spraying operations, combined with the concern about polluting the environment

with insecticides, led, in the 1970s, to a revival of interest in traps (Jordan, 1986). Killing a small percentage of the adult flies over a sustained period is an effective control strategy largely because of the low reproductive rates of tsetse flies (Williams *et al.*, 1993; Vale *et al.*, 1985). The area over which control needs to be maintained in order to be effective depends on the movement patterns of the tsetse flies.

The biconical trap was the first of many new designs of traps made from blue and black cloth (Williams *et al.*, 1993). The use of host odours such as acetone, various phenols found in cow urine and octenol that attract tsetse flies increases the efficiency of traps by over thirty times (Vale, 1993; Williams *et al.*, 1993 ; Green, 1994). Recent efforts have concentrated on retaining the efficiency while reducing the cost, and simplifying the design so that they can be constructed in rural areas (Williams *et al.*, 1993).

Targets are sheets of cloth, usually black or blue, sometimes with netting attached, which are impregnated with insecticides (Vale, *et al.*, 1988a). They are baited with odours used in traps. Targets are simpler and cheaper than traps and can be more effective for some species (Williams *et al.*, 1993).

1.7. Study Area

1.7.1. Location

Our field studies were carried out in the Luangwa Valley of Zambia at Kakumbi Research Station (13° 34'S, 31° 46'E and altitude 550m). The experiments were done within 15 km radius from the research station. The research station is located in the Game Management Area (GMA), while the research sites were in the South Luangwa National Park (map 5 in Annex 2) in undisturbed natural habitat where tsetse flies had no shortage of animal hosts.

1.7.2. Climate

The research station is found in Region I of the Zambia agro-climatic Zones. It has the typical Region I climatic conditions of rainfall 700-800 mm per annum with high chances of long dry spells in the rainy season and temperature ranges of 17 - 42°C during the year.

1.7.3. Vegetation

The vegetation is deciduous Savannah wood land. The main woodland type is Mopane woodland with *Colospermum mopane* as the main tree type. Other vegetation types are riverine vegetation with *Diospyros mespiliformis* as the major tree type. Thickets of mainly *Combretum spp.* are also common. Open dambos are widely spread throughout the area.

1.7.4. Landuse and Other Economic Activities of the Luangwa Valley

The area near the main gate to the South Luangwa National Park is heavily settled by people while the rest of the Luangwa valley is sparsely populated. Most people engage in subsistence agriculture while others work in the nearby government departments, lodges and tourist camps. The small scale farmers grow sorghum, maize and cotton. There are very few domestic animals which are mainly goats, pigs and chickens. Eco-tourism is the major commercial activity of the area. The national park is very rich in fauna especially large ungulates. The most common animals include, impala, puku, zebra, water buck, elephant, bush buck, kudu, buffalo, common duiker, hippo, wildebeest, giant eland, sable antelope, roan antelope, giraffe, lion, leopard, yellow baboon, civet, velvet monkey and other smaller mammals and reptiles like crocodiles and monitor lizard are also common. Other common mammals are warthog, bush pig, reed buck, grysbok, wilddog, hyena and hare.

1.8. Structure of the Thesis

The research work presented in this thesis is mainly on *Glossina brevipalpis*. The subjects under study on this fly were diverse and hence to cover and represent the research protocols and the findings more clearly, it was necessary to separate each aspect of the study hence each chapter deals with a specific subject reflected in the objectives.

CHAPTER 2

Sampling Methods for *Glossina brevipalpis* Newstead and Other Tsetse Flies (Diptera: Glossinidae)

2.1. Introduction

Most of the available information on the biology and control of tsetse flies (Diptera: Glossinidae) has been produced through the study of only a few tsetse fly species in each of the *Morsitans* and *Palpalis* groups (Challier & Laveissierre, 1973; Brightwell *et al.*, 1991; Vale, 1993). We therefore, have very little information that is directly relevant to other species such as *G. brevipalpis* Newstead and *G. longipennis* Corti which belong to the *Fusca* group. The situation arose because it is generally believed that the *Fusca* group played only a minor role in the transmission of bovine trypanosomosis. However, recent outbreaks of the disease among cattle in KwaZulu/Natal, South Africa have suggested that *G. brevipalpis* could be an important vector (Kappmeire, 1997).

The paucity of information on the *Fusca* group has since started to be redressed in the last decade, with studies of *G. longipennis* in Kenya (Brightwell *et al.*, 1991; Kyorku, *et al.*, 1990) and *G. brevipalpis* in South Africa (Kappmeier, 1997; Nevill *et al.*, 1993). Despite the fact that *G. brevipalpis* occurs in Zambia, little has been done to study this species

because the fly has seldom been caught by the sampling methods which are developed for catching *G. morsitans morsitans* Westwood, *G. m. centralis* Muchado, and *G. pallidipes* Austen. As a prelude to studying *G. brevipalpis* in Zambia it was considered necessary to first of all find appropriate and effective methods that can give large catches of the fly.

This study was an attempt to identify satisfactory means of sampling *G. brevipalpis* by comparing the catches of various sampling devices and techniques in an area of Zambia where previously this species had been found only occasionally among the large catches of *G. m. morsitans* and *G. pallidipes*. Annex 3 shows some of the sampling systems used in the study. These are epsilon trap, F3 trap, biconical trap, NGU2G trap and artificial refuge.

2.2. Study Site

This study was performed in two blocks of riparian woodland. One was two square kilometers and the other was three square kilometers and are both adjacent to the Luangwa river and its ox-bow lakes; a distance of about 15 kilometers to the west of Kakumbi Research Station. The woodland had a sparse undergrowth of bushes with a nearly continuous canopy provided by large semi-evergreen trees. The dominant trees were *Diospyros mespiliformis* Hochst (Ebenaceae) and *Acacia albida* Delile (Mimosoideae). Other tree species included *Ficus sycomorus* L.

(Moraceae), *Combretum microphyllum* Klotzch (Combretaceae) and *Trichilia emetica* Vahl (Meliaceae). Various wild animals, including buffalo, hippopotamus, elephant, warthog and bush buck were abundant at both these study sites. The riparian woodland area gets flooded from January to April and becomes cool and dry from May to August and then very hot and dry from September to November. The daily maximum temperatures can go up to 42°C in the shade during the hot dry season.

2.3. Materials and Methods

2.3.1. Experimental Design.

Comparisons between baits employed a Latin square design of baits by sites by days, or using randomised blocks in which each bait was operated at a particular site for one day in each of a group of adjacent days. All fly rounds and traps were used with odour attractants according to the method employed by Torr (1990.). The odours used consisted of acetone or butanone released at 200-500 mg/h from an open bottle, and a 1:4:8 mixture of 3-n-propyl phenol: 1-octen-3-ol : 4-methyl phenol released at a total of one to two mg/h from a polyethylene sachet. The dispensers were carried by men during the Man Fly Round (MFR) or fixed to the screen on the Black screen Fly Round (BFR) or placed on the ground 20 cm downwind of the traps. Most catches at certain baits and times were too small for the analysis of variance to be done and hence the results were presented as totals, with chi-squared

tests for the homogeneity of the sex and species composition of catches. When the term "significant" is used it implies significance at the 0.05% level of probability.

2.3.2. Fly Rounds.

A path of 1500 m long, describing a roughly oval shape, was cut through the two kilometer square block of woodland. Stop-marks were made on the path at 100 m intervals. The first fly rounds were carried out early in the morning between 05.00 and 08.00 h and then between 16.30 and 18.30 h in the evenings. Epsilon traps were used in the experiment as controls and were set at 500 m intervals along the fly round route. The traps were operated for twelve hours of day light and were emptied every morning at 08.00 h. For the MFR,*two men walked along the foot path and stopped for two minutes to catch tsetse flies on every 100 m mark. The BFR was the same as the MFR except that the men carried a pole between them from which a vertical screen of black cotton cloth, 120 cm long and 90 cm wide was suspended. At each stop-mark on each fly round, the men used hand nets to catch flies found on men and the screen, as well as on nearby ground and vegetation. This study was carried out in the hot dry season (August to December).

2.3.3. Traps.

Epsilon, NGU2G, F3 and biconical traps were used. Each trap was operated individually for twelve hours of the day light at sites that were about 200 m apart. The sites were cleared of grass and shrubs within four meters, and had no bushes closer than four meters.

2.3.4. Traps and Electric Screens

Prompted by the experience with other tsetse species (Vale, 1993), it was suspected that the failure of traps to catch many *G. brevipalpis* was not only due to the traps attracting few flies, but rather due to the failure of the traps to catch many of the flies that may have arrived near them. This investigation was further carried out between August and September of 1997 by using Epsilon traps with electric nets.

2.3.5. Fly-rounds with Electric Nets

Given the fact that trap catches are reported to severely underestimate the attractiveness of the traps (Vale, 1998), it was expected that the catches on the fly rounds could also underestimate the attraction of tsetse flies to moving objects. This problem was investigated in the period September to October of 1997 by fixing an electric net to a pole carried between two men.

2.3.6. Odour

Between January 1994 and December 1995 each of the four types of traps were used for 20 days with each of the six odour treatments consisting of no odour, acetone alone at 150, 300, 700 and 3000 mg/h, and the combination of 3000 mg/h of acetone with the standard mixture of 1-octen-3-ol and phenols. Another experiment was also performed to see whether tsetse fly catches would improve with greater doses of odour and with butanone being used instead of acetone. During the rainy season (December 1994 to March 1995), cool dry season (April to July 1995) and hot dry season (August to November 1995) an Epsilon trap was operated for 25 days with each of 18 odour treatments consisting of no ketone, or acetone or butanone at 150, 3000, 12000 and 25000 mg/h, all with and without the standard mixture of 1-octen-3-ol and phenols

2.3.7. Refuges

In order to evaluate their tsetse fly trapping efficacy of artificial refuges (Vale, 1971) were placed in dense shade with their entrances facing south or north, in order to avoid direct sunlight penetrating the interior. Each refuge was operated individually at sites about 200 to 300 m apart. At each inspection time of the refuge, the flies inside were caught by placing a cone of netting over the entrance and disturbing the flies with a stick until the tsetse flies flew into the cone where they were trapped in a

clear-glass bottle with a non-return baffle. The cone was then removed to allow more flies to enter the refuge.

2.3.7.1. Preliminary Study

A preliminary study was run for 16 days in October and November of 1993 using two box refuges, each consisting of an open-ended 200 litre metal drum painted black inside and insulated with earth (soil) and shaded by a grass thatched box measuring 80 x 80 x 120 cm. The aim of this experiment was to test the artificial refuges as a sampling system for tsetse flies.

2.3.7.2. Seasonal Use of Refuges

The two box refuges were emptied at 13:00 hours on eight to ten days of each month between July 1996 and June 1998. The data obtained for these study years were combined to obtain an average daily catch for each calendar month.

2.3.7.3. Diurnal Use of Refuges

In October 1997 the two box refuges were emptied at hourly intervals between 06.00 and 18.00 h to find out the diurnal use of artificial refuges.

Several experiments were performed to elucidate the principles of refuge design and to determine whether modification to the box refuge (Annex 3e) would improve its cost-effectiveness. Effects of insulation, shade, volume and colour inside of a refuge were tested in experiments which compared their effectiveness with or without them. The experiments ran concurrently for 32 days between 24th September and 4th November, 1997.

2.3.7.5. Refuges as Larviposition Sites

To investigate the role of refuges as larval deposition sites, experiments where some refuges were provided with gray metallic trays 85 cm long, 55 cm wide and 10 cm deep, filled with river sand were employed. The sand consisted of white grains about one to two millimeter in diameter, and it was provided with a 50% cover of leaf litter. The trays were placed on the ground, in the darkest part of the interior of the refuges and were inspected weekly to check for Pupae.

2.4. Results

2.4.1. Fly-Rounds

A total of 147 trap days, 100 BFR and 88 MFR were run. The highest catch for tsetse flies irrespective of species was 1616 and only 9 (0.6%)

were *G. brevipalpis*, 1088 (67.3%) were *G. pallidipes* and 519 (32.1%) were *G. m. morsitans* from epsilon traps (Table 2.1). For *G. m. morsitans* and *G. pallidipes* the results (Table 2.1) accord with expectation (Buxton, 1955) in that the catches of male *G. m. morsitans* did not differ much between the MFR and BFR but the proportion of *G. pallidipes* in the catches and the proportion of females of both species increased significantly in the order MFR, BFR and epsilon traps. All *G. brevipalpis* were caught from epsilon traps. The null hypothesis of an even proportional representation of *G. brevipalpis* in the trap and fly round catches was rejected at $p < 0.01$.

Table 2.1.: Catches using epsilon trap, Man Fly Round (MFR) and Black screen Fly Round (BFR) in the hot dry season.

Tsetse fly species	Epsilon trap (%)			MFR (%)			BFR (%)		
	male	female	total	male	female	total	male	female	total
<i>G. m. morsitans</i>	144 (27.8)	375 (72.2)	519 [32.1]	750 (94.0)	48 (6.0)	798 [97.3]	666 (84.3)	124 (15.7)	790 [92.9]
<i>G. pallidipes</i>	273 (25.1)	815 (74.9)	1088 [74.9]	14 (63.6)	8 (35.4)	22 [2.7]	30 (49.2)	31 (50.8)	61 [7.2]
<i>G. brevipalpis</i>	6 (66.7)	3 (33.3)	9 [0.6]	0	0	-	0	0	-
Sampling system total	423	1193	1616	764	56	820	696	155	851

Note: () species/sex/method percentage

[] species/method percentage

2.4.2. Traps

Since results from the above work suggested that traps seem to offer a better means of sampling *G. brevipalpis*, studies were made to find out whether catches could be improved by using different tsetse fly traps (epsilon, NGU-2G, F3 and biconical). However, a total number of 33 (12 males and 21 females) of *G. brevipalpis* were captured from the traps in comparison to 782 (234 males and 548 female) *G. m. morsitans* and 10,226 (3,082 male and 7,144 female) *G. pallidipes* during all the three seasons of 1997. Due to the small number of *G. brevipalpis* by each trap (33 compared with 10226 for *G. pallidipes*) it becomes unnecessary to proceed with further analysis of the results.

2.4.3. Traps with Electric Nets

The catch for *G. brevipalpis* using traps alone was only 0.7% (2 flies) of the total catch but when an electric screen was placed besides the trap, the numbers of *G. brevipalpis* caught by the electric net increased (162), the proportion increased from 0.7 to 19.9%. The results from traps with electric nets (Table 2.2) confirm that traps attract many *G. brevipalpis* but fail to capture them efficiently.

Table 2.2.: Tsetse flies catches of epsilon trap alone and epsilon trap with an electric net besides.

Treatment	Catch distribution	<i>G. brevipalpis</i> (%)	<i>G. pallidipes</i> (%)	<i>G. m. morsitans</i> (%)
Trap alone		2 (0.7)	124 (45.1)	149 (54.2)
Trap plus electric net	Trap	0 (0.0)	90 (43.3)	118 (56.7)
	Electric net	162 (19.9)	223 (27.4)	430 (52.8)

2.4.4. Fly-Rounds with Electric Nets

Table 2.3.: Capture efficiency of fly rounds to tsetse flies attracted.

Bait	Tsetse fly catches (%)		
	<i>G. brevipalpis</i>	<i>G. pallidipes</i>	<i>G. m. morsitans</i>
Electric screen	65 (9.1%)	46 (6.4%)	606 (84.5%)
BFR	15 (6.4%)	5 (2.1%)	213 (93.6)%
MFR	14 (9.3%)	2 (1.3%)	134 (89.33%)

The proportion of *G. brevipalpis* attracted by the three methods of capturing was stable though the numbers of tsetse flies caught were higher using the electric screen fly rounds (Table 2.3). This indicates that

the MFR and the BFR underestimate the numbers of tsetse flies attracted for all three species.

2.4.5. Odours

The results of all the odour experiments were pooled to bring out the huge discrepancy in the catches especially for *G. brevipalpis*. The total catches of *G. m. morsitans* and *G. pallidipes* were 42 (0.67%) and 6217 (99.11%) respectively. Only 14 (0.22%) (four males and ten females) of *G. brevipalpis* were captured, indicating that change in the odour used did not improve the attractiveness of traps for *G. brevipalpis* as was seen and reported elsewhere for *G. pallidipes* (Torr, 1990).

Despite the poor catches of *G. brevipalpis*, pooled results for all the experiments on odour and all the trap types were consistent with the expectation that odour attraction can improve catches for certain tsetse fly species. The average catches per 100 trap days were 0.08 with no odour, 0.16 with ketone alone, and 0.38 with the combination of ketone, 1-octen-3-ol and phenols.

2.4.6. Refuges

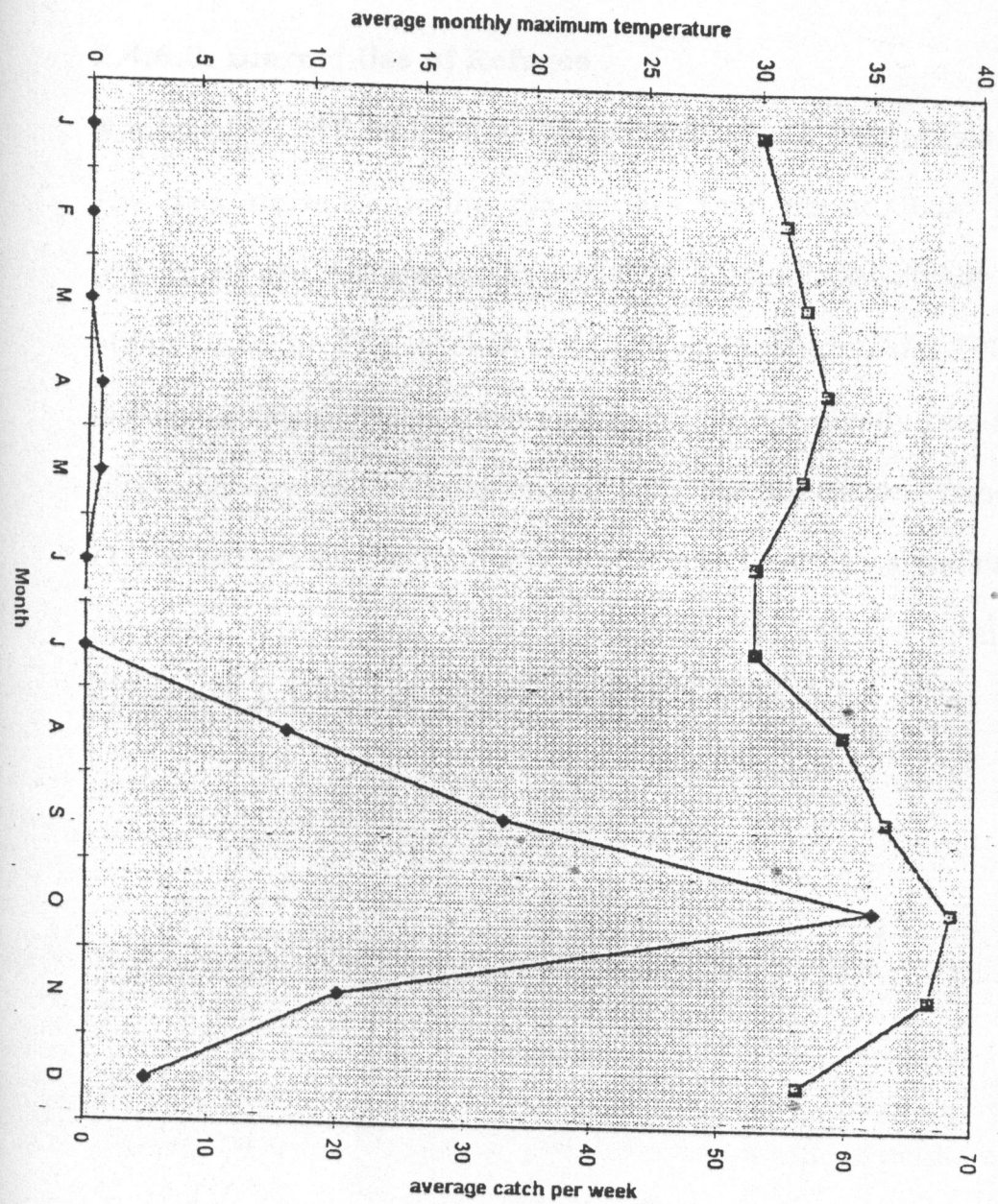
2.4.6.1. Preliminary Study

When refuges were tested for the first time in 1993, the catches of *G. m. morsitans* and *G. pallidipes* were 59 and 63, respectively. The catch of *G. brevipalpis* was 53, (27 males and 26 females) representing a remarkably high proportion on the overall catches. It seemed that refuges offered a promising method of sampling *G. brevipalpis*, so prompting more research.

2.4.6.2. Seasonal Use of Refuges

The total catches in the study were 1046 *G. brevipalpis*, consisting of 580 males and 496 females. For *G. m. morsitans* and *G. pallidipes* the catches were 2100 and 1802, respectively, and they followed the seasonal pattern evident for *G. brevipalpis*. Catches depended markedly on the daily maximum shade temperature, as measured in a Stevenson screen (Figure I). No *G. brevipalpis* were caught when the temperature was less than 32°C, but catches rose rapidly as temperature increased above that level and *vice-versa* (correlation coefficient $r=0.92$). Hence, the catches were high in the hot dry weather of August to December, with a peak in October. There was a small shallow peak in the warm wet period of April and May.

Figure 1: Seasonal refuge catch

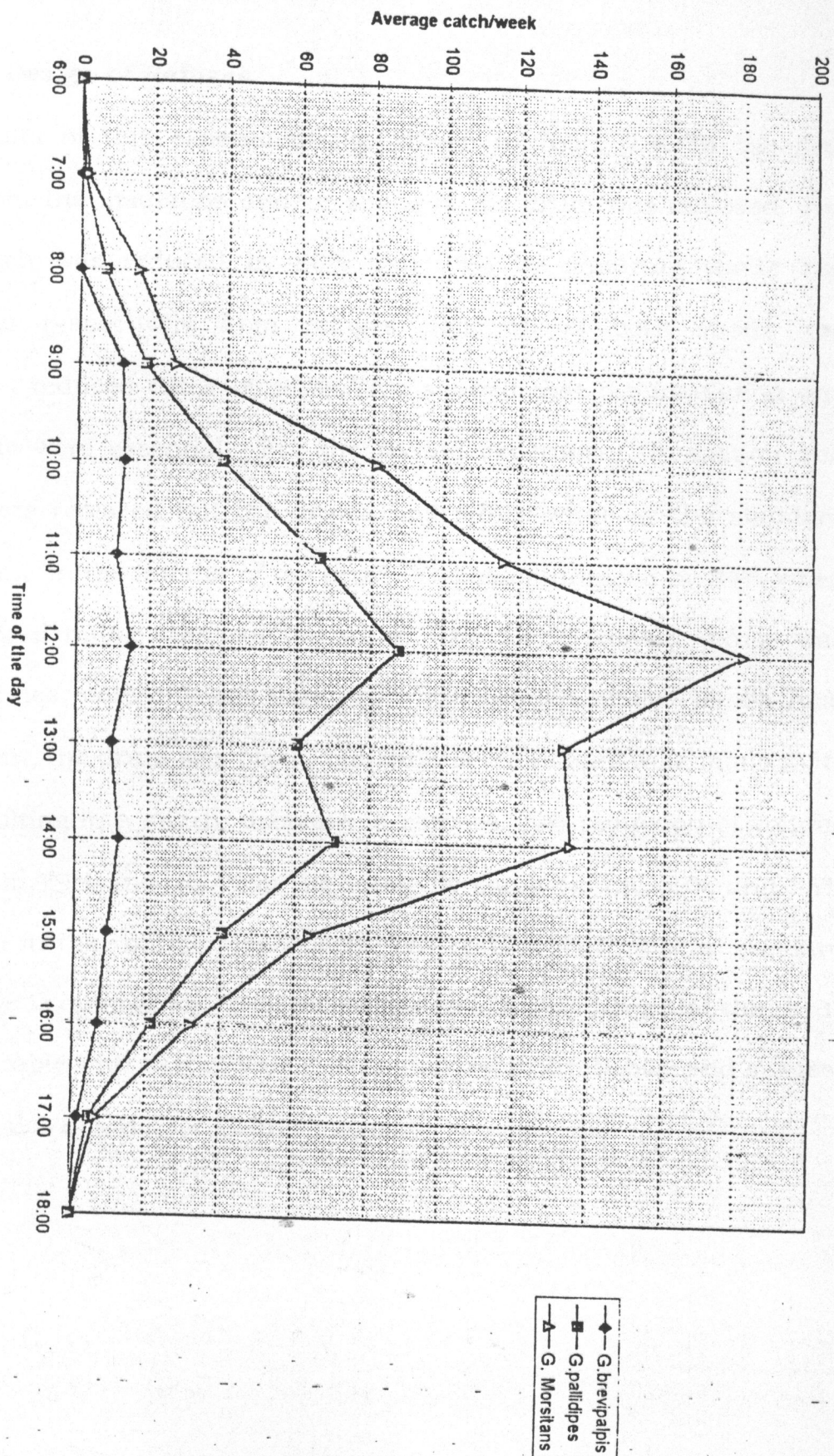


—■— Av. max. temp
—◆— Av. Catch per week

2.4.6.3. Diurnal Use of Refuges

A total of 91 (47 males and 44 females) of *G. brevipalpis* were caught. The flies started entering the refuges from about 08.00 h to 09.00 h (Figure II) and continued to enter in a seemingly steady stream until about 16.00 h. The catches of 414 *G. m. morsitans* and 793 *G. pallidipes* showed a distinctive pattern consistent with a steady rise in entry rates until mid-day and a sharp drop during the afternoon. The proportion of *G. brevipalpis* in the total catch of tsetse flies was significantly reduced between 10.00 h and 14.00 h, compared to catches in either the four hours before or the four after.

Figure II: Diurnal refuge pattern



2.4.6.4. Design of Refuges

The results of this experiment are shown in table 2.4. In the first experiment the insulation and/or shade of the drum was removed. The total catch was reduced by 80% and 78% by removing shade and insulation respectively from refuges. Removal of both shade and insulation reduced catch by 99%. This showed that the insulation and the shade were essential. For the second experiment, insulation and shade were replaced but the drums had either white or the standard black paint on the interior of the drum. The catch dropped by 84% when the artificial refuge was painted white. The black painted refuge was several times more efficient than the white painted refuge. In the final experiment, the drum was black, but various reductions were made to its size, resulting in a sharp decrease in catches. The reduction of volume resulted in 79% and 100% in the tsetse fly catch for the 50% and 75% reduction in the volume respectively. The results confirm that standard box refuge is optimal for *G. m. morsitans* and *G. pallidipes* and the result is comparable with that of Vale (1971) working in Zimbabwe and suggest that it is also optimal for *G. brevipalpis*.

2.4.6.5. Larval Deposition in Refuges

In a total of 16 weeks between September to mid-December 1997 only 30 Pupae were collected. All the pupae were for *G. m. morsitans*.

2.5. Discussion

Although the diurnal pattern of refuge entry of *G. brevipalpis* may differ slightly from that for *G. m. morsitans* and *G. pallidipes*(figure II), it was observed from the studies of seasonal occupation and refuge design that the three species respond to refuges in much the same way; each species has a response threshold of about 32°C (figure I) and each accumulates most in dark, capacious refuges that are shaded and insulated. Refuges offer a powerful means of sampling all three species but it is unfortunate that the temperature threshold is so high for the tsetse flies to enter, hence effectively limiting the usefulness of refuges to about three months in the hottest season, even at a generally warm place such as the Luangwa Valley. Moreover, as with *G. pallidipes* (Vale, 1971), refuges are poor means of studying the larval deposition of *G. brevipalpis*.

For most of the year it is necessary to sample with baits other than refuges, but the standard fly rounds and traps give inadequate catches of *G. brevipalpis*, even with the use of odours that seem to increase catches several times for other tsetse fly species. However, the results with the

electric nets show that there is nothing fundamentally wrong with the mobile and stationary baits as attractants. Instead, the problem is that the hand-nets and trapping mechanisms catch only a very small proportion of the many *G. brevipalpis* attracted. In this regard, *G. brevipalpis* seems qualitatively similar to *G. m. morsitans* and *G. pallidipes* (Vale, 1974b), but quantitatively distinct in that the inefficiencies of capture are in an order of magnitude greater with *G. brevipalpis*.

For the time being, electric sampling devices must be the mainstay of sampling methods for studying the behaviour and ecology of *G. brevipalpis* for most of the year. Such techniques applied to *G. brevipalpis* in ways similar to those used for *G. m. morsitans* and *G. pallidipes* (Vale, 1993) are likely to produce an improved trap for research and surveys, and an effective design of odour-baited target for the control of *G. brevipalpis*. The fact that odours added to traps and fly rounds did not result in large catches of *G. brevipalpis* does not mean that odours are ineffective for this species. It is necessary to repeat the work with catching devices that are more likely to catch the odour attracted flies.

Since *G. brevipalpis* seems to be much more localised than *G. m. morsitans* and *G. pallidipes*, its control may be relatively easy. However, there is the associated problem that the researcher could soon catch-out

the population in the study area if electrocuting devices are used extensively. These devices kill the flies they catch, whereas tsetse flies caught by traps, refuges or hand-nets can be marked and released, so they do not deplete the population. This emphasizes the need to improve trap efficiency to the point where traps are suitable research tools. It also emphasizes that *G. brevipalpis* is the prime candidate for the first use of the radar tracking device now being developed to study tsetse behaviour (Riley *et al.*, 1996). *Glossina brevipalpis* is the largest tsetse fly and may therefore, be best able to carry a trackable tag. Moreover, the fact that it occupies limited patches of woodland with a sparse undergrowth would enhance the convenience and effectiveness of radar tracking.

CHAPTER 3

The Distribution of *Glossina brevipalpis*, *G. m. morsitans* and *G. pallidipes* in Three Major Vegetation Types of the Luangwa Valley

3.1. Introduction

Successful control of tsetse flies (*Glossina* spp) largely depends on having an idea of where tsetse flies occur (Vale, 1994). The term distribution is defined as where creatures occur (Southwood, 1980; Andrewertha & Birch, 1954). In this study, distribution shall be defined in terms of relative trap catches. Where no tsetse flies are caught represents absence; low catches represent low density and high catches represent high density. The distribution and abundance of organisms, central topics in the long-continuing ecological debate about population regulation (Clark *et al.*, 1967; Dempster, 1983; Hassell, 1985), assume particular importance in the case of pests and vectors of disease. If the control of these organisms is to be achieved by scientific rather than empirical means, it must be based on a proper understanding of the natural dynamics of the population under field conditions (Rogers & Randolph, 1986). Tsetse fly control methods have relied to a larger extent though crudely, on the distribution of tsetse flies over time and space (Vale, 1994; Buxton, 1955). In the game elimination method of tsetse fly

control, wild animals were killed so that tsetse flies would have no food hence the flies would either starve or move to areas where hosts were readily available. In ground spraying, the insecticide was deposited on suitable resting sites in more ideal vegetation types capable of carrying higher tsetse fly populations (Buxton, 1955). With the advent of the bait technology, traps and targets are planted in suitable tsetse fly habitats and ensures the use of higher target densities against high tsetse fly populations (Vale *et al.*, 1986).

Tsetse fly surveys are usually carried out during those periods in the year when the tsetse fly population are expected to be high enough, however, this depends on the tsetse fly species involved (Jordan, 1986).

The distribution of tsetse flies over time and space is therefore a major factor in understanding the ecology of tsetse flies which in turn, is an important consideration in tsetse fly control and survey operations. The distribution of *G. m. morsitans* and *G. pallidipes* has been well documented (Vale *et al.*, 1986; Phelps & Vale, 1978; Hargrove, 1981a; Rogers & Randolph, 1986), but the distribution of *G. brevipalpis* has remained largely unknown (FAO, 1992). The main objective of this study was therefore, to elucidate the distribution over time (seasons) and space (vegetation type) of *G. brevipalpis* relative to *G. m. morsitans* and *G. pallidipes*.

3.2. Study Sites

The study sites were located in the South Luangwa National Park as indicated in map 5 (Annex 2 i.e. Luangwawafwa riverine vegetation, mopane woodland and thicket woodland). The vegetation in the Luangwa valley can be divided into three major types:

(a) Mopane woodland: This is an open type of woodland. The main tree species is *Colophospermum mopane*. Other tree species include *Adansonia digitata* and *Combretum microphyllum* creepers. This area is very thick during the rainy season (December to March).

(b) Thicket vegetation: This is a double canopy vegetation type which is always thick. The top canopy is formed by *Combretum appiculatum* trees while the second canopy is formed by *Combretum microphyllum* creepers. In some areas, *Cordyla africana* trees tower over both canopies.

(c) Riverine vegetation: This double canopy vegetation type occurs along the Luangwa river banks and along its lagoons and tributaries. The major tree species found here is *Diospyros mespiliformis* which forms the top canopy. Other tree species are *Acacia albida*, *Trichila emetica*, *Kigeria africana* and *Beuhenia thonningi* which form the lower canopy. This vegetation type is restricted to areas prone to seasonal flooding which

occurs during the rainy season. It therefore has very little undergrowth and is almost devoid of grass in the dry season.

3.3. Materials and Methods

In each of the vegetation types, three epsilon traps and one artificial box refuge were set. The traps were operated for up to 15 days each month from September 1997 to August 1998. The same sites were maintained for the whole study period. The traps were set up for 12 hours of day light and the cages were emptied every day in the morning between 06:00 h and 08:00 h. The tsetse flies were killed by exposure to direct sunrays for twenty minutes. The artificial refuges were run from September to December, 1997. The refuges were checked every day at 12:00 h. The flies in refuges were collected using a cone of netting as described in Chapter 2. All flies collected were categorized into species and sex. The measure of the relative distribution in the experiment were the average catch per trap or refuge in each vegetation type. The distribution over space was measured by comparison of the catch in the different vegetation types while the distribution over time was deduced from seasonal trends. The seasons are (i) Hot dry season (August to November); (ii) Rainy season (December to March) and (iii) Cool dry season (April to July).

3.4. Results

3.4.1. Traps

The highest catches in traps were for *G. pallidipes*, followed by *G. m. morsitans* and least was *G. brevipalpis* (Table 3.1). More female tsetse (70%) were caught in traps than males (30%). The catch for *G. brevipalpis* was generally low in the traps. There was a significant difference in the catches of *G. brevipalpis*, *G. m. morsitans* and *G. pallidipes* in the three vegetation types ($\chi^2_{\text{calculated}} = 18931.22$, $\chi^2_{\text{critical}} = 18.47$ at $df=4$, $p=0.001$). More *G. brevipalpis* was caught in the riverine vegetation than the Mopane vegetation ($t_{\text{calculated}}=12.92$, $t_{\text{critical}}=4.44$ at $df=11$ and $p=0.001$). The *G. brevipalpis* catch in the riverine vegetation was also significantly higher than the catch in the thicket vegetation type ($t_{\text{calculated}}=12.67$, $t_{\text{critical}}=4.44$ at $df=11$ and $p=0.001$). The total catch was highest in the thicket (26995) followed by the mopane (26505) and least was the riverine (4290). There were significant differences in catches (all species) between riverine and mopane woodland ($t_{\text{calculated}}=3.34$, $t_{\text{critical}}=3.11$ at $df=11$ and $p=0.01$). The catch (all species) in riverine vegetation was also significantly lower than the thicket vegetation ($t_{\text{calculated}}=6.03$, $t_{\text{critical}}=4.44$ at $df=11$ and $p=0.001$). There was no significant difference between catches (all species) from the mopane and thicket vegetations ($t_{\text{calculated}}=0.05$, $t_{\text{critical}}=2.20$ at $df=11$ and $p=0.05$).

For each *G. brevipalpis* caught, 19, 12686 and 12912 of *G. pallidipes* were caught in the riverine, mopane and thicket vegetations respectively. For *G. m. morsitans*, 6, 593 and 585 were caught for each *G. brevipalpis* captured in riverine, mopane and thicket vegetations respectively (Table 3.2). In all vegetation types, the catch of *G. brevipalpis* was lower than for the other species (Table 3.1). There were no significant differences in the catch of *G. m. morsitans* between vegetation types [riverine versus mopane ($t_{\text{calculated}}=0.94$, $t_{\text{critical}}=2.20$ at $df=11$ and $p=0.05$); riverine versus thicket ($t_{\text{calculated}}=0.78$, $t_{\text{critical}}=2.20$ at $df=11$ and $p=0.05$) and mopane versus thicket ($t_{\text{calculated}}=0.14$, $t_{\text{critical}}=2.20$ at $df=11$ and $p=0.05$)].

The catch of *G. pallidipes* was significantly higher in both thicket ($t_{\text{calculated}}=5.998$, $t_{\text{critical}}=4.44$ at $df=11$ and $p=0.001$) and mopane ($t_{\text{calculated}}=3.35$, $t_{\text{critical}}=3.11$ at $df=11$ and $p=0.01$) woodlands relative to the riverine vegetation but there was no significant difference between the *G. pallidipes* catch in the thicket and mopane ($t_{\text{calculated}}=0.04$, $t_{\text{critical}}=2.20$, $df=11$, $p=0.05$). See Table 3.3.

In the riverine vegetation, where the catch for *G. pallidipes* was lower than the other two vegetation types, there was a significant difference with catch of *G. m. morsitans* ($t_{\text{calculated}}=2.69$, $t_{\text{critical}}=2.20$, $df=11$, $p=0.05$).

See Table 3.3.

Table 3.1: Summary of annual catches of tsetse flies from traps

Vegetation type	<i>G. brevipalpis</i>	<i>G. pallidipes</i>	<i>G. m. morsitans</i>	Totals
Riverine	160	3106	1024	4290
Mopane	2	25317	1186	26505
Thicket	2	25824	1169	26995

Table 3.2: Species ratios in different vegetation types

Vegetation type	Relative ratios of distribution/tsetse catches			
	<i>Gmm</i> : <i>Gp</i>	<i>Gb</i> : <i>Gp</i>	<i>Gb</i> : <i>Gmm</i>	<i>Gb</i> : <i>Gp</i> : <i>Gmm</i>
Riverine	1 : 3	1 : 19	1 : 6	1 : 19 : 27
Mopane	1 : 21	1 : 12659	1 : 593	1 : 12659 : 593
Thicket	1 : 22	1 : 12912	1 : 585	1 : 12912 : 585

Table 3.3: Average epsilon trap catches in each vegetation type per day.

Season	Vegetation type	<i>G. brevipalpis</i>	<i>G. pallidipes</i>	<i>G. m. morsitans</i>
Hot dry (Sep-Nov)	Riverine	0.8	37.40	11.54
	Mopane	0.02	79.13	10.98
	Thicket	0	136.75	12.54
Rainy (Dec-Mar)	Riverine	1.4	13.03	5.00
	Mopane	0.02	266.83	5.96
	Thicket	0.02	204.18	3.51
Cool dry (Apr-Aug)	Riverine	1.4	16.82	6.41
	Mopane	0	128.83	5.48
	Thicket	0.02	139.33	6.29

3.4.2. Artificial Refuges

The catches in refuges were highest for *G. pallidipes* followed by *G. m. morsitans* and least was *G. brevipalpis* (Table 3.4). During this experiment, the catches in refuges were generally low in all the vegetation types. Due to the lower catches, no test of significance was carried out. *Glossina brevipalpis* was only captured in the riverine vegetation. It was also noted that more *G. pallidipes* were caught using artificial refuges in the mopane woodland than in any other vegetation type while more *G. m. morsitans* were caught in the thicket vegetation.

Table 3.4: Summary of tsetse flies caught from artificial refuge (Number in parenthesis is the average catch per day).

Vegetation type	Days run	Catch		
		<i>Gb</i>	<i>Gp</i>	<i>Gmm</i>
Riverine	40	21 (0.5)	19 (0.5)	14 (0.4)
Mopane	40	0	60 (1.5)	13 (0.3)
Thicket	40	0	15 (0.4)	51 (1.3)

* *Gb*- *G. brevipalpis*, *Gp*- *G. pallidipes*, *Gmm*- *G. m. morsitans*

From the catches in traps and refuges, *G. brevipalpis* was concentrated in the riverine vegetation throughout the year (Tables 3.1, 3.3 and 3.4). The highest concentration of *G. m. morsitans* occurred in the dry season for all vegetation types, whereas the catch for *G. brevipalpis* did not show any seasonal variation or peak as can be seen in the comparison of the catch for *G. brevipalpis* between the rainy and hot dry seasons ($t_{\text{calculated}}=0.39$, $t_{\text{critical}}=3.18$, $df=3$, $p=0.05$); hot dry and cool dry seasons ($t_{\text{calculated}}=0.06$, $t_{\text{critical}}=3.18$, $df=3$, $p=0.05$); cool dry and rainy seasons ($t_{\text{calculated}}=0.61$, $t_{\text{critical}}=3.18$, $df=3$, $p=0.05$).

From trap catches, *G. pallidipes* were concentrated in the thicket in the hot dry season while in the rainy season more *G. pallidipes* was found in

mopane woodland though the difference was not significant from the thicket. It was also noted that more *G. pallidipes* were found in the thicket during the cool dry season than the mopane though the difference was not significant. The seasonal trend in the tsetse fly catches in the riverine vegetation is as presented by figure III where the population of all the three species is low in the rainy season but increases in the cool dry season through the hot dry season and starts dropping in November for *G. pallidipes* and *G. m. morsitans* while for *G. brevipalpis* the reduction in catches starts in September.

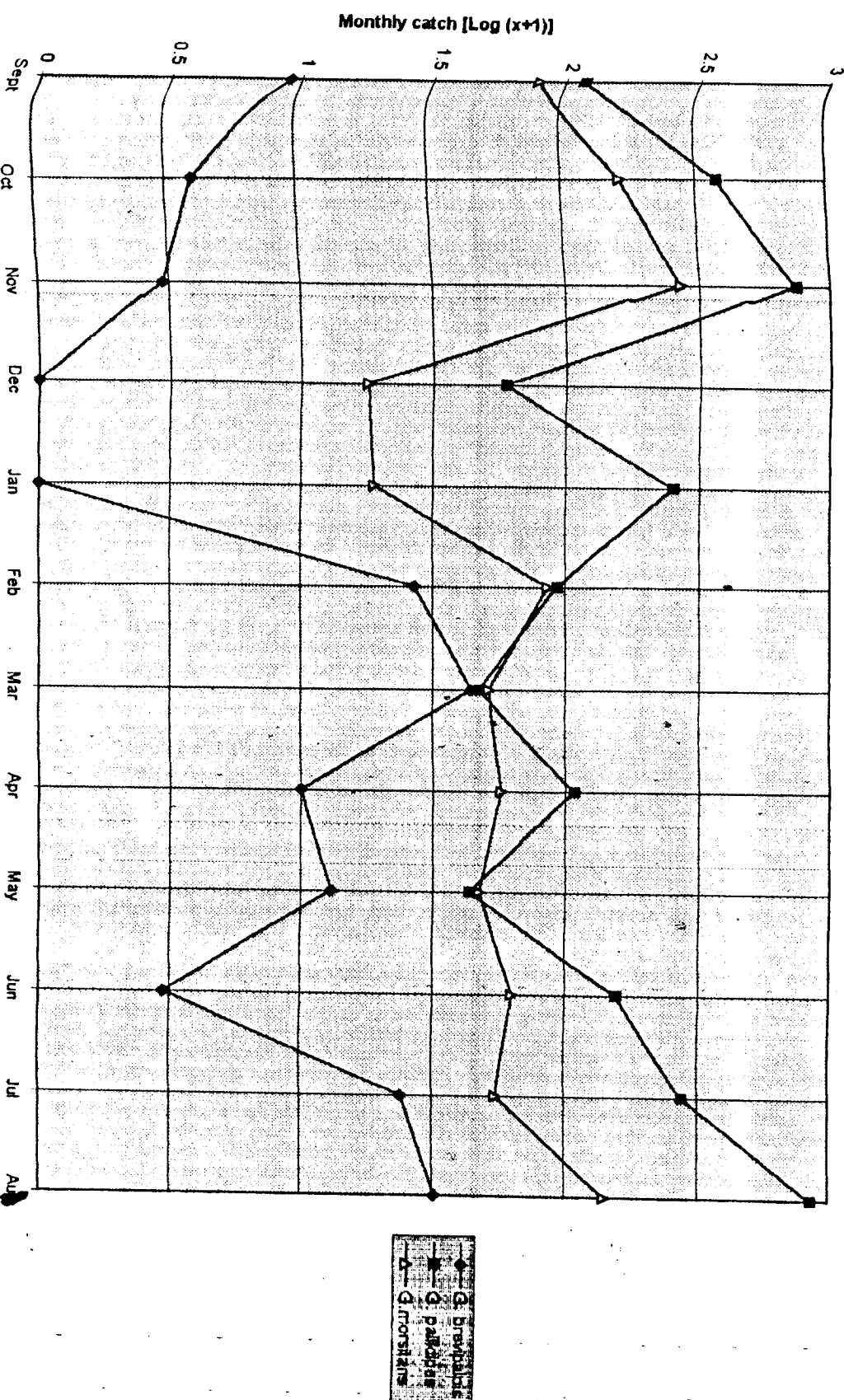


Figure III: The seasonal trend of tsetse fly catches in the riverine vegetation

3.5. Discussion

If tsetse flies were tortoises we could learn much of what we need to know about their ecology and behaviour by simply watching them (Vale, 1998). However, the flies are too small and fast to be observed easily (ibid). Video cameras have been useful aids in the last few years but they can record tsetse fly only in limited situations (Gibson *et al.*, 1991). We are forced to deduce important aspects of tsetse fly biology indirectly by studying catches at baits. It is difficult enough to deduce the number, condition and behaviour of the flies that arrive near baits (Vale, 1993), let alone an assessment of these aspects of the flies in the population as a whole. Tsetse flies are regarded to be most abundant where they are most caught (Pilson & Pilson, 1967). If catches decline between seasons, it is commonly assumed that tsetse fly populations have declined. However, there are three problems to these interpretations. Firstly, fly catches do not only depend on the abundance of the flies but also on their responsiveness to visual and olfactory stimuli, and we know little about the responsiveness in various seasons and vegetation types. Secondly, the catches in one vegetation type relative to another further depends on the type of bait employed (Vale, 1971). Thirdly, catches can even vary substantially from site to site within a given vegetation type (Saunders, 1964; Vale, 1998). The afore-mentioned factors have not been adequately explained and elucidated.

Our difficulties in interpreting catch size are argued by problems of interpreting catch composition. The relative numbers of various species, sexes and physiological categories in catches can not be taken as indications of the composition of the wild population if no allowance is made for the responsiveness of the populations components. We know little about such responsiveness in the field and must make assumptions about it. It therefore becomes difficult to deduce much more from the results than is assumed (Vale, 1998).

The catch in a trap is a representation of the tsetse fly population in an area, efficiency of the trap, effectiveness of the baits used and other factors like mobility of the tsetse flies which is dependent on host availability in the area and species of tsetse flies involved. The interaction of these factors also affects the catch at baits. Assuming equal attractiveness of traps in the different vegetation types and different seasons, then the trend of tsetse fly catches in traps represent a crude measure of the population in the areas. Catch is especially difficult to interpret in terms of the sample composition (sex ratio, age, nutritional status) as a representation of the population due to sampling biases; Trap catches are biased towards *G. pallidipes*, and often more females are captured. Trap catches are also biased towards older tsetse flies (Vale, 1998).

Recent work by Vale (1998) has brought to light the effects of vegetation on trap catches. Since mopane vegetation is the only vegetation which is relatively flat, has clear sites, with no tall trees nearby and clear of bushes near traps, it was expected that the highest catch of *G. m. morsitans* and *G. pallidipes* should be highest in this vegetation type. This was not observed. The other vegetation types always had overhead canopy cover and hence shade most of the day which should have depressed the catch further but to the contrary the highest overall catch was observed in the thicket vegetation. It appears that though in open vegetation types bushes and shade affects catch negatively, in thicket vegetations with canopies the effects of bushes and shade near traps needs further investigation. Moreover, since the thicket vegetation and to some extent the riverine vegetation leads to less odours being released since the dispensers are almost always in the shade, the catch was supposed to be reduced further but contrary to expectation, this was not observed. The optimal trap siting rules suggested by Vale (1998) though helpful in open vegetation types, need more investigation so that the factors at play in thickets and vegetation with broken terrain and canopies are incorporated. It could also be related to host availability where more preferred hosts are available in thicker vegetation types than open ones.

Despite all these problems in interpreting catches at baits in different vegetation types, the data collected has important implication in control of tsetse flies. Since there is no better method of studying the causes of seasonal variations in catch in different vegetation types, then we have to rely more on the use of traps, artificial refuges and fly rounds as opposed to electric screens (targets), which are not only expensive but require specialists in their maintenance. More over, the use of electric screens is also limited to dryspells. We could in fact be discouraged to even attempt to discuss the distribution of tsetse fly species such as *G. brevipalpis* on which the basic sampling tools are just being developed. However, with the available sampling tools, limited as they are, it has been shown that this fly is limited to riverine vegetation and rarely ventures to other vegetation types in the Luangwa Valley and South Africa (Nevill, *et al.*, 1993). *Glossina m. morsitans* and *G. pallidipes* are relatively well distributed in the three vegetation types.

Due to the restricted habitat of *G. brevipalpis*, control of this species can be easily and efficiently achieved because the restriction in the habitat is very pronounced. With an efficient catching-out system like an S-target (1 m X 1 m black cotton cloth flanked by 0.5 m X 0.5 m clear netting on both sides) can effectively control this fly. The possibility of catching out *G. brevipalpis* during research is high because the flies' habitats in the

Luangwa valley are small hence the use of electric nets, which kill tsetse flies on capture, should be kept to a minimum.

CHAPTER 4

Dispersal and Daily Displacement of *Glossina brevipalpis* in a Riverine Woodland of the Luangwa Valley.

4.1. Introduction

The movement of tsetse flies has attracted considerable attention and consequently we are informed relatively well about their dispersal (Glasgow, 1963). The above statement may not be true for the *Fusca* group of flies (FAO, 1992) which includes *G. brevipalpis*. A lot of research has been conducted on *G. m. morsitans* and *G. pallidipes* dispersal and daily displacement (Phelps & Vãle, 1978; Hargrove, 1981ab; Vale *et al.*, 1984). We must note that in some works on tsetse flies, a specialized use of the word "dispersal" is found and is illustrated below. It often happens that tsetse flies are living in one particular type of vegetation, such as the riverine thickets along a water course, and that cattle are being grazed on the nearby grassland. Although tsetse flies can not live permanently in the open grassland, they can fly out for a certain distance into the surrounding areas and back into their usual habitat. This spread of tsetse flies from their true habitat varies according to the species of *Glossina* involved, and also according to the season, and to the nature of the area into which they are spreading. This apparent tsetse fly movement is a matter of great importance where cattle are kept in the

vicinity of tsetse fly infested areas. Such spreading of tsetse flies is sometimes referred to as “dispersal”, especially in the writings of Lewis (1950, 1951). It is a kind of dispersal but in this study, dispersal is used in a wider sense to include movements inside as well as outside tsetse fly habitat. The daily displacement is thus defined as the average distance covered by tsetse flies per day. The distance is expressed linearly, although tsetse fly movement is random and mainly circular (Brady & Griffiths, 1993). Such tsetse fly movement has a bearing on the interpretation of catches which is a measure of the populations based on the sampling systems used. This is because catches from any sampling system are dependent on among other things, the available population of tsetse flies and their mobility (Vale, 1993). The daily displacement of *G. morsitans* and *G. pallidipes* was reported to be 700-800 m and 900-1000 m per day respectively (Phelps & Vale, 1978). However, there is no information in the literature about the displacement of *G. brevipalpis*. It is with this background therefore, that the following experiment was carried out.

4.2. Study Sites

The experiments were carried out in a riverine woodland area called Nkhomawafwa (Map 5 in Annex 2) along the Luangwa river. The woodland is composed of mainly *Diospyros mespiliformis* trees and it is bordered by the Luangwa river on the west and an open mopane

woodland on the east. The area is prone to seasonal flooding during the rainy season.

4.3. Materials and Methods.

Artificial refuges were used for the capture of *G. brevipalpis* according to the procedure followed in chapter 2. Eight (8) artificial refuges were used in the study. The refuges were arranged roughly in a circle of 300 meters radius which covered the habitat width. One artificial refuge was placed in the center while the other seven were in the perimeter of the circle. The refuges were between 200 m to 300 m apart and the distance between refuges was dependent on the availability of naturally shady positions. Refuges four, five and seven were outside the riverine habitat hence were used as markers to detect any *G. brevipalpis* flying out of their riverine habitat. All the other refuges were in the riverine habitat. The flies were collected once a day between 12:00 h and 14:00 h (during the hottest time of the day when tsetse flies rest in refuges) for three weeks in October of 1998. All the tsetse flies were caught with a cone of netting placed at the mouth of the refuge, a procedure developed by Vale (1971). The flies were each uniquely marked with Parker® artists paint and released immediately on the sites of capture. The marking cord consisted of four colours (red, white, yellow and blue). The flies' dorsal view of the abdomen and thorax was divided into ten positions and each fly was marked with the four colours to give each fly a unique identity

(Annex 4). The movement of the tsetse flies was recorded on subsequent recaptures and their daily movement noted. Simple calculations of average distance covered over the days taken was used as a measure of displacement.

4.4. Results

A total of 318 *G. brevipalpis* were captured in refuges and were uniquely marked and released. A total of 21 *G. brevipalpis* (Table 4.1) were recaptured mainly from refuge one (seven flies) and refuge six (twelve flies) giving a recapture rate of 6.8%. One fly each was recaptured from refuge three and refuge eight. One *G. brevipalpis* recaptured from refuge six could not be identified fully because the red mark could not be found. Refuge two did not record any catch of *G. brevipalpis* even though it was in the riverine habitat. No *G. brevipalpis* was captured or recaptured from refuges four, five and seven which were outside the riverine habitat. At the beginning of the fourth week, there was some heavy rains and the catches in refuges ceased. The daily displacement for the recaptured *G. brevipalpis* was estimated at 31 m per day for the twenty identified recaptured flies. The displacement was calculated by considering the distance from the refuge of first capture to the refuge of recapture divided by the number of days it took between first capture and recapture. This calculation method only takes into consideration the radial movement away from point of first capture and not total displacement. Twenty

percent of the recaptured (identified) flies were recaptured from the refuges of first capture.

Table 4.1: Summary of the mark recapture experiment for *G. brevipalpis*.

Refuge no. of first capture	Fly number	Refuge no. of recapture	Interval in days between first capture and recapture	Distance between refuges (m)
6	14	6	7	0
6	79	1	9	300
6	105	6	6	0
6	204	1	1	300
6	230	1	1	300
1	26	6	10	300
6	268	1	2	300
6	280	3	1	200
6	205	6	1	0
8	313	6	1	100
1	156	6	8	300
1	147	6	8	300
8	284	6	1	300
1	296	8	1	300
1	188	6	7	300
1	262	6	6	300
?	?	6	?	?
1	7	1	23	0
1	7	1	1	0
6	229	1	17	300
1	202	6	17	300

4.5. Discussion

The recapture rate of 6.8% was lower than in previous initial experiments (Maseko, unpublished data) where recapture rates were as high as 24% in similar vegetation types. Even the recapture rate from the refuge of the first capture was also much higher (60%) than the 20% observed during this experiment. There does not seem to be any cause for the discrepancy. The circle of artificial refuges used in the experiment was small compared to the large square (16 sq. miles) experiment of Jackson (1946). It should be more interesting to use a less restricting experimental design where the riverine vegetation is sampled outside the initial cycle of artificial refuges. However, the estimated daily dispersal of 31 m per day is very low in comparison to other tsetse flies. The estimated daily dispersal for *G. m. morsitans* and *G. pallidipes* are 700-800 m and 800-900 m, respectively (Phelps & Vale, 1978). The estimated daily dispersal for *G. brevipalpis* of 31 m is slightly higher than that estimated for *G. f. fuscipes* by Bursell (1970) of 183 m per week (26 m/day).

The estimated 31 m is the average displacement per day it took the fly to cover the distance between refuges of first capture and refuge of recapture while the measures for *G. m. morsitans* and *G. pallidipes* are an average of all movements a fly makes in a day. Since dispersal in tsetse flies can be viewed as a series of discrete daily steps taken in a random

direction (Bursell, 1970; Rogers, 1977) and since *G. brevipalpis* is confined to isolated patches of riverine woodland, it could have covered comparable distances but because of the restricted habitat, the radial distance covered per day is seen to be short. The habitat is restricted as seen from the experiment since all refuges outside the riverine woodland did not catch any *G. brevipalpis*. The effect of the restricted riverine habitat on the movement of *G. brevipalpis* needs more investigation as it has serious implications on other ecological questions on the fly especially on matters relating to host location and host preference.

Since *G. brevipalpis* does not respond to artificial baits and odours as well as *G. m. morsitans* and *G. pallidipes* (Chapter 2) and that the habitat is restricted then how do the flies find their hosts? Since most animal hosts are mobile and only a few such as the bush buck are resident in the riverine habitat, does it entail a very restricted host range or is it that hosts come to the fly as against the flies looking for the host? Could this then explain the inability to attract lots of these flies using artificial baits and odours?

While this experiment is only a preliminary study, studies on the host preference should take center stage in the understanding of the ecology of *G. brevipalpis*. Further, studies on the dispersal pattern should be extended where larger habitats are available and other sampling systems

are available since refuges are very restrictive in terms of season (can only be used in the hot dry season) and positioning is dependent on naturally available shade. Artificial refuges, though, have the advantage that they are able to attract all these flies' sexes and age categories as they occur naturally. And since refuges do not depend on odour attractants/baits, they are more likely to help understand the movement of tsetse flies without the effects of hunger and mating which affect catches at baits. *Glossina brevipalpis* was observed not to be affected by the paint as it flew off immediately after making like all the other tsetse flies do.

With the restricted habitat and the lack of wide foraging habits, control of this tsetse fly species may be easily achieved using artificial refuges lined with insecticide treated clothes which have been tested in other experiments to have the ability to retain tsetse flies (Maseko, unpublished data).

CHAPTER 5

Composition and Assessment of Infection Rates of Catches of *Glossina brevipalpis*, *G. m. morsitans* and *G. pallidipes* from Epsilon Traps and Artificial Refuges.

5.1. Introduction

A population sample which is collected without bias can give a good indication on the composition of that population. The composition of insect pests especially in terms of age, nutritional status, developmental stage (egg, larvae, pupae and adult) is a very important factor in an effort to control the pests (Southwood, 1980). Effective control of arthropods is best done at the most vulnerable stage of their development. In tsetse flies, only the pupae and adult stage are available for control due to their adenotrophic viviparity nature of reproduction where the egg and the larvae stages both occur in the female fly. The female of most insect species produces many eggs and then abandons them to their tenuous fortunes; but the female tsetse fly produces only one egg every ten days and guards its development very closely (Nash, 1969; Jordan, 1986; Vale *et al.*, 1985; FAO, 1980). The egg is retained in the uterus and after hatching the larva is fed on a material secretion until it is fully grown, by which time it weighs as much as the mother (Glasgow, 1963; Saunders, 1960). After finding a shaded spot the female extrudes the mature larvae

which immediately burrows to safety, carrying in its mid-gut the food its mother has provided to take it through its pupal life and to allow it to hunt its first meal as a newly emerged adult. This slow but sure method of reproduction is the reason for another peculiarity that sets the tsetse fly apart from most other insects - its stable population dynamics (Vale *et al.*, 1985).

Females usually mate within a day or so after emerging from their pupal case, the sexes generally meet when the female is about to or is actually in the process of taking her first blood meal. The females mate once and retain the sperms in the spermathecae and are released into the oviduct every time a female tsetse fly ovulates (Jordan, 1986).

While it is known that female tsetse flies live longer than males both in the laboratory and in the field, actual life-spans in the field vary tremendously (Jordan, 1986). The life span of the average female is critical as she must live for some 36 days to produce more than the two off-springs that must be produced if the species is to survive. In some places and at some time of the year, especially the hot dry season, it is certain that the average female does not survive long enough to produce sufficient off-spring to ensure the survival of the species and population decline occurs. At other climatically more favourable times of the year, surplus off-spring are produced allowing the population to recover but

even under laboratory optimal conditions, reproduction of twelve larvae by a female is exceptional (140 days) (*ibid*). Male tsetse flies normally live up to 46 days when optimal weather prevails (FAO, 1980).

Tsetse fly feeding interval is a hotly debated issue (Groenendijk, 1996). Some authorities believe tsetse flies feed on regular three to four day intervals (Rogers, 1977; Randolph & Rogers, 1978, Randolph *et al.*, 1991) while others believe that tsetse flies are opportunistic feeders and feed whenever they have an opportunity without recourse to regularity (Hargrove & Parker, 1993).

In estimating population mortalities from standing age distribution it is assumed, among other things, that the samples used are unbiased with respect to age (Caughley, 1977). Mortality in a sampled population of tsetse flies can be estimated from the ovarian age distribution (Saunders, 1962; Challier, 1965; Challier & Turner, 1985; Dransfield *et al.*, 1985; Rogers *et al.*, 1984; Turner, 1987; Van Sickle & Phelps, 1988). Females which have not ovulated, and which are thus less than nine days old, are excluded from such calculation on the ground that they are under represented in most trap samples. It is arbitrarily assumed that there is no sampling bias with respect to any older flies. However, there is no evidence to support this assumption but there is reason to suppose that

it may be false for at least *G. m. morsitans* and *G. pallidipes* (Hargrove, 1991).

Tsetse fly populations observed at Rekomitje (a tsetse fly search station in the Zambezi Valley, Zimbabwe) in terms of species composition, diurnal activity patterns and age structure were apparently dependent on the sampling method used (Hargrove, 1991). It was important therefore to investigate this observation for *G. m. morsitans* and *G. pallidipes* in comparison with *G. brevipalpis* in the Luangwa valley of Zambia.

Effective control of tsetse flies shall increasingly depend on our understanding of the ecology of the flies as in bait technology (Vale *et al.*, 1988a). Since our understanding of the ecology of insect populations is dependent on the sampling system used, with all the inherent sampling biases, efforts should be made to understand the population composition of tsetse fly species under such biased sampling methods.

5.1.1 Age Determination

There are two major methods of estimating age in tsetse flies; wing fray analysis (for both males and females) and ovarian analysis (for females). Both methods dependent on the physical and physiological changes that occur as the tsetse fly grows. These techniques were originally developed to deal with *G. m. morsitans* and *G. swynnertoni* but have now been

adopted to estimate the age of other tsetse fly species as well (FAO, 1980).

5.1.2. Wing Fray Analysis.

When a fly emerges from the pupa, the wings spread out to their full size and harden; the margin of the wing is perfect at this stage, without tears or irregularities. But later the wings become torn, particularly along the trailing (rear) edge of the wing, because during the daily activity the fly may brush against vegetation and the animals it feeds on, or may fight with other flies or may be attacked by predators. The wings therefore become more tattered with age, so that a very old fly has wings with badly torn edges.

5.1.3. Ovarian Analysis

In early studies of the pupipara such as *Melophagus ovinus* L Pratt (1899 cited in Saunders, 1960) and *Hippobosa longipennis* F. capensis by Detinova, 1955, cited in Saunders, 1960), found the sequential development of ovarioles starting from the right ovariole followed by left then right and left again to complete the cycle. In a study of the ovulation cycle of *Glossina palpalis* (Robineau-Desvoidy), Mellanby (1937) established that the first egg is ovulated from the right ovary and subsequent eggs alternate from left to right throughout the life of the tsetse fly. Similar cycles were seen to occur in *G. m. morsitans* Westwood

and *G. swynnertoni* Austen (Mellanby, 1937). It has now been shown that two ovarioles are present in each ovary (Saunders, 1960).

Ovarian analysis involves dissection to examine the ovaries and uterus. Although it is a more complicated method than the wing fray method because of the tsetse fly dissection involved, the age of individual tsetse flies can be estimated while the wing fray analysis estimates the age of the sample collected.

5.2. Materials and Methods

5.2.1. Fly Collection and Handling

There were two sources of data for this study:-

(a) Five epsilon traps baited with acetone, octenol, 3-n-propyl phenol and 4-methyl phenol and five artificial refuges were deployed in the riverine vegetation along the Luangwa River (Luangwawafwa area see map 5 in Annex 2). This is a habitat for *G. m. morsitans*, *G. pallidipes* and *G. brevipalpis*.

(b) Three other epsilon traps which were set in each of the following vegetation types: (i) riverine, (ii) mopane and (iii) thicket. These traps were used in the data collection of the seasonal study experiment in Chapter 3.

The experiment was run for up to 15 days in a month from September 1997 to August, 1998. The traps were run for 12 hours of day light and

flies were collected in the morning between 06:00 hr. and 08:00 hr. Flies were immediately placed in cool boxes with ice packs and transported to the laboratory where they were immobilized by keeping them at a temperature of 4°C in a refrigerator for one hour before dissection.

Tsetse flies from artificial refuges were collected as described earlier (Chapter 2) and stored in cooler boxes and treated as for the flies from the traps. The refuges were only run during September to December of 1997. All the flies from artificial refuges were dissected.

Only five *G. pallidipes* were dissected from each trap while all *G. m. morsitans* and *G. brevipalpis* were dissected. The *G. pallidipes* were restricted so that comparable numbers to the other species were collected as opposed to random sampling of all the three species in a trap catch because by far more *G. pallidipes* would have been dissected since the majority (>90%) of the trap catches were *G. pallidipes*..

5.2.2. Dissections

The wings and legs were cut off from the immobilised flies. There are six wing fray categories used for both male and female tsetse flies. For the details of the categories of the wing fray categories guide on the wing tatter and the calculation method see Annex 5. The proboscis, mid-gut and proventriculus, and salivary glands were examined under 0.9-1%

saline solution at magnification of X400 using a compound microscope (See annex 6 for full procedure of dissection). The method of estimating the physiological age used was that suggested by Saunders (1960) and categorized by Challier (1965) and improved by Hargrove, (1995) which is summarized in a table in Annex 6.

5.3. Results

5.3.1. Epsilon Traps and Artificial Refuges

Table 5.1. Results of the dissection data from artificial refuges and traps

Method of capture	Number dissected			Species ratio	Sex ratio	Age (days)		Infection rates (%)			
	<i>Gb</i>	<i>Gp</i>	<i>Gm</i>	<i>Gb:Gp:Gm</i>	Male:Female	Male	Female	<i>Gb</i>	<i>Gp</i>	<i>Gm</i>	Poole d
Artificial refuges	689	174	137	5:1.3:1	1:2.2	55	32	3.3	2.2	4.4	3.3
Epsilon traps	67	405	221	1:6:3.3	1:3.1	45	25	1.5	2.2	3.6	2.7

A total of 1000 tsetse flies captured from artificial refuges were dissected. Out of the total flies dissected, 33 (3.3%) were positive for trypanosomes. Nineteen (57.6%) of the positive flies were found with trypanosomes in the mouthparts only. The other 14 (42.4%) of the positive tsetse flies were found to have trypanosomes in the mid-gut and mouthparts. No fly had trypanosomes in the salivary glands.

A total of 693 tsetse flies were dissected from epsilon traps in the riverine vegetation. Nineteen (2.7%) of the tsetse flies from the epsilon trap were positive for trypanosomes. Ten (52.6%) were positive in the mouthparts only while the other nine (47.4%) were positive in the mouthparts and midguts. No fly had trypanosomes detected in their salivary glands.

There was a higher proportion of *G. brevipalpis* in artificial refuges (68.9%) than in epsilon traps (9.7%). The proportion of *G. pallidipes* in the refuge catch (17.4%) was lower than the proportion of this species in the epsilon trap catches (58.4%). The proportion of *G. m. morsitans* in the epsilon traps was 31.9% which was higher than that of the artificial refuges (13.7%).

The age distribution of the two samples is detailed in Table 5.2 for females and Table 5.3 for males.

Table 5.2. The age distribution of female tsetse flies in artificial refuges and epsilon traps.

Ovarian category	Artificial Refuges (%)	Epsilon traps (%)
0	4.2	5.2
1	11.3	8.8
2	12.4	13.6
3	18.2	16.9
4	22.4	24.0
5	16.8	17.3
6	10.4	10.6
7	4.2	3.6

Table 5.3: The age distribution of male tsetse flies in artificial refuges and epsilon traps.

Wing category	Artificial refuge (%)	Epsilon traps (%)
1	3.2	4.6
2	27.4	20.9
3	42.8	34.6
4	24.9	29.0
5	3.3	7.6
6	1.3	3.2

The age distribution for both male and female tsetse flies from artificial refuges and epsilon traps are not significantly different from each other.

5.3.2. Composition of Epsilon Trap Samples from Riverine, Mopane and Thicket Vegetation Types.

Table 5.4. Results of tsetse fly dissections in riverine, mopane and thicket vegetation

Vegetation type	Number dissected			Species ratio	Sex ratio	Age (days)		Infection rates (%)			
	Gb	Gp	Gm	Gb:Gp:Gm	Male:Female	Male	Female	Gb	Gp	Gm	Poole
Riverine	72	367	302	1:5.1:4.2	1:2.0	45	22	1.4	1.9	1.7	1.8
Mopane	0	1221	96	0:13:1	1:2.0	46	22	-	3.3	4.2	3.4
Thicket	0	1395	120	0:12:1	1:2.0	43	26	-	1.6	1.7	1.6

A total of 741 tsetse flies were dissected from epsilon traps in riverine vegetation. Of the total flies dissected, 13 (1.8%) were positive for trypanosomes. Of the 13 positive flies, 8 (61.5%) were positive in the mouthparts only while the other 5 (38.5%) were positive in the mouthparts and midguts.

A total of 1,317 tsetse flies were dissected from the epsilon traps in mopane woodland. Of all the tsetse flies dissected, 45 (3.4%) were positive with trypanosomes. There were 36 (80%) samples positive in the mouthparts while the other 9 (20%) were positive in the mouthparts and midguts.

There were 1,515 tsetse flies dissected from epsilon traps in the thicket vegetation. A total of 24 (1.6%) tsetse flies captured were positive for trypanosomes and of these, 23 (95.8%) were positive in the mouthparts while the other one (4.2 %) was positive in the mouthparts and midgut.

The age distribution of the flies are presented in Table 5.5 and 5.6.

Table 5.5. Age distribution of female tsetse flies from Epsilon traps in the riverine, mopane and thicket vegetation types.

Ovarian category	Riverine (%)	Mopane (%)	Thicket (%)
0	2.4	4.8	4.3
1	13.7	15.0	12.7
2	20.6	13.2	13.5
3	23.4	16.4	16.8
4	22.6	18.1	18.9
5	11.3	16.7	16.6
6	4.0	10.0	11.4
7	2.2	5.7	5.7

Table 5.6. Age distribution of male tsetse flies from Epsilon traps in the riverine, mopane and thicket vegetation types.

Wing category	fray	Riverine (%)	Mopane (%)	Thicket (%)
1		14.0	14.6	5.4
2		35.5	29.6	23.8
3		26.3	30.0	32.3
4		14.6	15.9	24.6
5		6.3	8.4	9.2
6		3.2	1.5	4.6

5.4. Discussion

The species composition of tsetse fly samples collected by different methods have been studied by many scientists (Pilson & Pilson, 1967; Vale, 1974a; Hargrove, 1991; Harley, 1967; Jackson, 1946). By collecting samples from the field, scientists want to know how representative the

sample is of the population (Southwood, 1980). The various methods of collection of tsetse fly field data have been evaluated. Man fly rounds, black screen fly rounds and mobile electric screens have been found to be biased towards the capture of more male and generally young tsetse flies (Vale, 1974b; Hargrove, 1991). In contrast the tsetse flies caught by traps (epsilon, F3, NGU, biconical and others) are said to be biased towards females and mostly older flies are collected (Vale, 1974b & 1998; Hargrove, 1991). It is also reported that the presence of man in the sampling system reduces the proportion of female tsetse flies and this is more pronounced for *G. pallidipes* than in any other tsetse flies species (Vale, 1982). Artificial refuges were reported to collect a more balanced sample for *G. pallidipes* and *G. m. morsitans* in Zimbabwe (Vale, 1971; Torr, 1994).

In this study, artificial refuges and epsilon traps were used to sample *G. brevipalpis*, *G. pallidipes* and *G. m. morsitans* in a riverine vegetation and for a seasonal variation study as detailed in Chapter 4. The species composition of samples from artificial refuges and epsilon traps were very different. The artificial refuges caught more *G. brevipalpis* than epsilon traps while *G. pallidipes* was captured more in epsilon traps. *Glossina m. morsitans* contributed the least to the total catch in the artificial refuge while *G. brevipalpis* was least in the epsilon traps. These results bring out a very important aspect on the interpretation of catches as measures

of tsetse fly population composition and abundance and that is you can not easily interpret catch composition as a reflection of population composition without recourse to the biases of the sampling systems.

The composition of the samples collected in terms of age was not significantly different in the artificial refuge catch and epsilon trap catch in this study. This finding is unlike the results reported by other authors (Vale, 1971; Hargrove, 1991). The interesting finding in this study is on the abundance interpretation of species catch by a given sampling system. Artificial refuges indicated a very high population of *G. brevipalpis* while the population of the other tsetse fly species are very sparse but then the trap catches are the exact opposite where *G. pallidipes* is the major component of the tsetse fly population while *G. brevipalpis* is sparsely populated. By both methods, the population of *G. m. morsitans* seems to fall between the two other species. The sample species composition becomes especially difficult to interpret when the catch from a sampling system where no tsetse fly is captured or where very few are captured as in the mopane and thicket vegetation where only two *G. brevipalpis* were captured from each vegetation type and during the same experiment, 25,000 *G. pallidipes* and over 1000 *G. m. morsitans* were captured. It may seem that *G. brevipalpis* does not inhabit the mopane and thicket vegetation types but it is likely that

relative to the riverine vegetation, there is less *G. brevipalpis* in the mopane and thicket vegetation types.

In all vegetation types, the age of the tsetse flies estimated by the ovarian analysis was always slightly higher than the age estimated by the wing fray analysis. There were flies in ovarian categories 3, 4, 5, 6 and 7 whose wing fray values were 1 and 2. This result though seemingly out of place, really reflects the different experiences of the individual tsetse flies that could result in the wing tear. In all vegetation types and sampling systems, infected flies were all older than 25 days which gives them enough time for the *T. vivax* and *T. congolense* to mature while *T. brucei* needs much older flies of over 35 days though no tsetse fly had the *T. brucei* infection.

The infection rates ranging from 1.4 - 4.4% in the whole study were lower than reported in other studies where infection rates ranged from 3.5 - 6.8% (Bealby *et al.*, 1996). The infection rates were highest in *G. m. morsitans* followed by *G. pallidipes* and least was *G. brevipalpis* and this accords the findings of Bealby *et al.*, (1996) for *G. m. morsitans* and *G. pallidipes*. There was no record on *G. brevipalpis* in the study by Bealby *et al.* (1996). Most of the infections were the mouthparts only type while the rest were for the mouthparts and midgut infection types which also accords with the other study (*ibid.*).

CHAPTER 6

Species Specific Identification of Trypanosomes in *Glossina brevipalpis*, *G. m. morsitans* and *G. pallidipes* by Polymerase Chain Reaction (PCR).

6.1. Introduction

Tsetse-transmitted African animal trypanosomes are elongate, usually slightly curved protozoan parasites, with a single nucleus. Their sizes range from 10-35µm long. The organelle of locomotion is the single flagellum which originates near the posterior end of the body of the trypanosome by a fold of the pellicle, the undulating membrane. In some trypanosome species such as *Trypanosoma congolense* the flagellum only extends as far as the anterior end but in others there is a free portion of the flagellum extending anteriorly. Near the basal body of the free flagellum is the dark staining kinetoplast. The diagnostic characters of the blood stream forms (trypomastigotes) of the various species of mammalian trypanosomes are the size and shape of the body, the position of the nucleus and kinetoplast and the length and form of the undulating membrane and flagellum, as observed in stained preparation of the parasites (Jordan, 1986; FAO, 1980; Nash, 1969, Mulligan, 1970, Buxton, 1955).

Trypanosoma species which undergo a cycle of development in the tsetse fly are called salivaria and belong to the family trypanosomatidae, order Kinetoplastidae. Four subgenera of the trypanosomes are recognized although the subgenus *Pycnomonas* which contains only one species *T. suis* is of very limited distribution and importance and is not discussed further. The various groups of tsetse transmitted trypanosomes are discussed separately below.

6.1.1. *Duttonella* (*Vivax* group)

There are two species of economic importance in this group.

(a) *Trypanosoma vivax*: This is a monomorphic species, 20-27 μm (average 22.5 μm) by 3 μm . The posterior part is distinctly broader and bulbous, the kinetoplast is large and terminal and the free flagellum is short, being 3-6 μm in length (Soulsby, 1986). The organism is very motile in fresh blood, moving very rapidly across the field, pushing red blood cells aside as it goes. It causes disease in domestic animals in Africa, Asia and South America. In Africa, the major vectors of the disease are tsetse flies (Soulsby, 1986; Losos & Ikede, 1972), while in Asia and South America, mechanical transmission by biting flies especially flies of the *Tabanus* spp. are implicated.

(ii) *Trypanosoma uniforme*: This is similar to *T. vivax* with an average length of 16 μm (12-20 μm) by 1.5 - 2.5 μm . The free flagellum is shorter

than that for *T. vivax*. It is transmitted cyclically by tsetse and occurs mainly in Uganda and the Democratic Republic of Congo (DRC) (Soulsby, 1986).

6.1.2. *Nannomonas (Congolense group)*

There are two species of economic importance in this group:

(i) *Trypanosoma congolense*: This is a small form 9-18 μm in length, and it is the smallest of the African trypanosomes. The posterior extremity is blunt and the kinetoplast is typically marginal, being some distance from the posterior end. Characteristically, there is no free flagellum though the tapering anterior extremity may, inadvertently, give the appearance of one. Although the undulating membrane is inconspicuous, the organism is active and it exhibits marked but non progressive movements in fresh blood. *T. congolense* is widely distributed in southern and parts of East Africa and it produces the most important pathogenic form of trypanosomiasis. It is transmitted cyclically by tsetse flies (Jordan, 1986; Nash, 1969, Mulligan, 1970, Soulsby, 1986).

(ii) *Trypanosoma simiae*: This is a polymorphic form resembling *T. congolense*. The majority of the trypanosomes are 16-24 μm , being long and stout with a distinct undulating membrane. About 7 % of the population tend to be long and slender with an inconspicuous membrane, and a few are short with a poorly developed undulating membrane. Transmission is mainly by tsetse flies though cases of

mechanical transmission by *Stomoxys* and *Tabanids* occur (Soulsby, 1986).

6.1.3. *Trypanozoon (Brucei group)*

There are three sub-species in this group:

(i) *Trypanosoma brucei brucei*: This is a polymorphic trypanosome with slender, intermediate, and stumpy forms occurring. The undulating membrane is conspicuous in all forms while the kinetoplast is sub-terminal and the tail pointed. The slender forms are 25-35 μm (average 29 μm) in length, have a long free flagellum and a pointed posterior end. The intermediate forms average 23 μm , the posterior end is more blunt and a free flagellum is present. The stumpy forms average 15 μm , the posterior end is broad, the kinetoplast is more terminal and the flagellum is lacking. Movement is snake like or wriggling, the organism seldom move out of the field. All forms may occur in the blood at the same time but usually only one form predominates. The species is widely distributed between 15° north and 25° south. Transmission is mainly by tsetse flies. Some mechanical transmission is also possible (Soulsby, 1986; Losos & Ikede, 1972).

(ii) *Trypanosoma brucei gambiense*: This is the cause of Gambian sleeping sickness (the trypanosomiasis of humans). It is morphologically indistinguishable from *T. b. brucei* found in animals. It is a polymorphic with slender, intermediate, and stumpy forms. *Trypanosoma b. gambiense* occurs mainly on the West Coast of Africa between the latitude of 15° north and 18° south and parts of Central and East Africa with lake Victoria as an eastern limit. Riverine tsetse flies are the major vectors. Domestic animals are not easily infected but if infection occurs it is not as severe as *T. brucei brucei* and *T. brucei rhodesiense* (Losos & Ikede, 1972).

(iii) *Trypanosoma brucei rhodesiense*: This is the cause of Rhodesian sleeping sickness of humans. The parasite is morphologically indistinguishable from *T. brucei brucei* and *T. brucei gambiense*. The organism is polymorphic showing all the three forms similar to other species. It is restricted to Southern Africa and parts of East Africa and around the shores of Lake Victoria. The major vectors are Savannah tsetse fly species. It results in mild pathogenicity in cattle, goats and sheep (Losos & Ikede, 1972)..

6.1.4. Trypanosomes in tsetse flies

Tsetse flies emerge from pupae uninfected by trypanosomes and hence can not transmit the parasite. When a tsetse fly takes a blood meal from

an infected animal, there is a chance that it can become infected. A small proportion of the trypanosomes in the blood of an animal are in a suitable condition and form to infest the tsetse fly; for instance, the stumpy forms of *T. b. brucei* are more capable of establishing infection in the tsetse flies. The trypanosomes undergoes a cycle of development in the fly which is unique to each trypanosome group (FAO, 1980). In general, the trypomastigote transforms into procyclics (without Variable Antigen Type [VAT]) before becoming metacyclics (with VAT) which are infective to mammalian hosts. It is reported that different salivarian trypanosomes follow different pathways in the tsetse fly habitat (FAO, 1980, Buxton, 1955).

6.1.4.1. *Duttonella*

Some trypanosomes taken up by the fly attach themselves to the walls of the food canal formed by the labrum and labium in the proboscis. They form colonies and multiply. In about ten days infective forms are produced that migrate to the hypopharynx and infect new hosts when tsetse feed on their next host. This cycle takes about 10 days (FAO, 1980).

6.1.4.2. *Nannomonas*

Most of the trypanosomes that are carried on to the gut survive. They then migrate forward to the food canal of the proboscis where they

multiply. Later infective forms reach the hypopharynx, and new hosts can be infected when the fly feeds. The cycle takes 12-14 days (FAO, 1980).

6.1.4.3. *Trypanozoon*

Trypanosomes are carried to the gut, later passing forward to the proboscis. From here they enter the hypopharynx and reach the salivary glands, where finally the infective forms are produced. The cycle takes 20-30 days (FAO, 1980).

6.1.5. Identification of Trypanosomes in the Tsetse Fly

Identification of trypanosomes in the tsetse fly has mainly relied on the location of motile trypanosomes in the fly according to the following categories (FAO, 1980):

- (1) *Duttonella* - if found only in the mouthparts.
- (2) *Nannomonas* - if found in the mouthparts and mid-gut.
- (3) *Trypanozoon* - if found in the mouthparts, mid gut and salivary glands.

6.1.6. Distribution of Trypanosome Infection Rates in the Tsetse Fly.

While it has been reported that trypanosome species affecting cattle occur in equal proportions of 1:1:1 for *T. vivax*: *T. congolense*: *T. brucei* respectively (Lubinga, 1996), the infection rates distribution in tsetse flies is still not clear. However, the predominant species is generally reported to be *T. vivax* followed by *T. congolense* and the least being *T. brucei* (Bealby *et al.*, 1996; Lubinga, 1996). One of the reasons for the apparent discrepancy in the infection rates distribution in tsetse flies and in cattle for *T. vivax*, *T. congolense* and *T. brucei brucei* could be attributed to the techniques used for the identification of the trypanosomes. Since most of the infections in cattle are transmitted by tsetse flies, and that we assume that all trypanosomes have an equal chance of establishment in the host, then the proportions of trypanosome species in the tsetse flies should also be expected to be well balanced, unless otherwise.

The main aim of this study was therefore, a) To develop a protocol for isolation of trypanosome DNA from infected tsetse fly parts, b) To identify at molecular level, the species of trypanosomes isolated from the mouthparts and the mid guts of proven infected tsetse flies.

6.2. Materials and Methods

6.2.1. Collection of Infected Tsetse Flies

Fresh tsetse flies were caught using standard procedures in the field. They were dissected to remove the mouthparts, mid guts and salivary glands which were examined under a microscope set at magnification of X240. Each of the parts were mounted on a slide and covered with a cover slip to which a solution of 0.1% saline was added. All the *G. brevipalpis* parts which were found positive were carefully transferred to a number four Whatmans® filter paper and were immediately frozen (-20°C) to preserve the DNA in the trypanosomes. To avoid contamination of the samples, forceps were washed and sterilized in boiling water after each use and that each pair of forceps was only used for a particular tsetse fly part.

6.2.2. Isolation and Purification of Trypanosome DNA

The *G. brevipalpis* specimen samples were pooled in batches of 5 for the midguts and 10 for the mouthparts. A composite sample made by collecting a few microliters from all the other samples (purified DNA before the PCR reaction) was used to run the PCR reaction to test for *T. simiae*. Dot blots of the specimens in each batch were punched out and soaked in 5 ml Phosphate Buffered Saline (PBS) in a 10 ml test tube for 24 hours at 4°C to allow the trypanosomes to detach from the tsetse fly parts and filter paper. This was followed by vortexing the test tube to

release the trypanosomes into the homogenate. Approximately 1 ml of the PBS - homogenate was recovered by decanting and this was then transferred to a 1.5 ml clean centrifuge tube. The homogenate was centrifuged for one minute at 500 rpm to allow coarse material (tsetse fly parts and filter paper) to settle at the bottom. The supernatant was decanted in to a new 1.5 ml centrifuge tube and followed by high speed centrifugation at 10,000 rpm for 5 minutes for the trypanosomes to collect as a pellet at the bottom of the centrifuge tube. The excess supernatant was drained off and fresh PBS (1 ml) was added to the trypanosome pellet and vortexed to allow the trypanosomes back into solution. The resulting solution was centrifuged at 10,000 rpm for 5 minutes to wash the trypanosomes which were then collected in a pellet form. The excess supernatant was drained off and the pellet vortexed in 100 μ l of PBS which served as the sample template DNA.

6.2.3. Polymerase Chain Reaction (PCR) Amplification

A slightly modified PCR amplification technique was carried out in a reaction of 25 μ l mixture (Solano *et al.*, 1995; Katakura *et al.*, 1997). The reaction mixture was prepared by adding 10 μ l template DNA, one Ready-To-Go Beads®, 1 μ l each of primers one and two and 13 μ l of sterilised distilled water to make the volume of 25 μ l. The PCR Ready - To - Go Beads when brought to a final volume of 25 μ l contains 1.5 units of Taq Polymerase, 10 mM Tris-HCl (pH 9 at room temperature), 50 mM KCl,

1.5 mM MgCl₂, 200 µM of each dNTP and stabilisers, including BSA (Pharmacia Biotec, 1996). The primers used were TCN1 (TCGAGCGAGAACGGGCACTTTGCGA) and TCN2 (ATTAGGGACAAACAASATCCCGCACA) for *T. congolense* Savannah subgroup; TCF1 (GGACACGCCAGAAGGTACTT) and TCF2 (GTTCTCGCACCAAATCCAAC) for *T. congolense* Forest subgroup; TBR1 (CGAATGAATASTTAASACAATGCGCAG) and TBR2 (AGAACCATTATTAGCTTTGTTGC) for *T. brucei*; TSM1 (CCGGTCAAAAACGCATT) and TSM2 (AGTCGCCCCGGAGTCGAT) for *T. simiae*; and TVW1 (CTGAGTCGCTCCATGTTGCCAC), and TVW2 (CCACCAGAACACCAACCTGA) for *T. vivax* (Kirchoff & Donnelson, 1993). The reaction mixture was overlaid with 20 µl of mineral oil and cycled in programmable heating blocks as follows. Incubation at 94°C for three minutes in an initial step followed by 40 cycles involving denaturation at 94°C for one minute, annealing at 55°C for one minute and extension at 72°C for one minute. The samples were incubated at 72°C for 7 minutes and then cooled at 4°C to stop the reaction.

To the PCR product, 2 µl of DNA loading buffer was added and 10 µl of the mixture was placed in 2% agarose gel stained with ethidium bromide in TAE buffer (40 mM Tris Acetate and 1 mM EDTA) at pH 8.

In the first well only 5 µl of DNA biomarker was added. The product was separated electrophoretically in the TAE buffer at 100 V until the dye had

covered half way the gel after approximately 40 minutes. The reaction was observed under Ultra-Violet (UV) light.

6.3. Results

6.3.1. Development of the PCR protocol

A protocol outlined in the method for the isolation, purification and running of the PCR up to the electrophoretic separation was established. The DNA amplification worked satisfactorily for the tsetse fly midguts, but not for the tsetse fly mouthparts where no amplification was observed.

6.3.2. PCR Amplification.

A total of 20 PCR amplifications were carried out on 85 positive *G. brevipalpis* midguts and 20 mouthparts. The results are presented in table 6.1.

Table 6.1. Results of PCR amplification for mid-gut (all other *trypanosoma* species and mouthparts (*T. vivax* only) for specimens

PCR	Number of batches	Positives	Negatives	Damages
<i>T. congolense</i> (Savannah)	7	7 (100)	0 (0)	0 (0)
<i>T. congolense</i> (Forest)	3	0 (0)	2 (66.6)	1 (33.3)
<i>T. brucei</i>	7	3 (42.9)	4 (57.1)	0 (0)
<i>T. simiae</i>	1	0 (0)	1 (100)	0 (0)
<i>T. vivax</i> (mouthparts)	2	0 (0)	2 (100)	0 (0)

* Number in parenthesis is percentage (%)

6.4. Discussion

A simple protocol has been developed to isolate trypanosomes from tsetse fly midguts using dot blots on filter paper for PCR amplification. The results open a way for a better understanding of the tsetse fly ecology by explaining an anomaly, especially in the identification of trypanosomes in the tsetse flies. The method was able to identify 100% *T. congolense* (Savannah) the most important and common trypanosome in Zambia. No *T. congolense* (forest) was found in the batches tested. This trypanosome is rare though it has been reported and may not have been in the batches used in this experiment (Bealby, *et al*, 1996).

It was interesting to note that 42.9% of the midgut positive batches carried *T. brucei* trypanosomes though all the tsetse flies used in the tests were negative for *T. brucei* from the microscopic examination results because there were no trypanosomes detected in the salivary glands. A composite sample made by collecting a few microliters from all the other samples (purified DNA before the PCR reaction) was not positive for *T. simiae*. It was also noted that no *T. vivax* was detected by PCR even though the flies used in this specific batches were from flies that were found positive only in the mouthparts by microscopic examination. There are three possible explanations for this; i) The *T. vivax* primers failed to amplify, similar failures were also observed by other workers (Katakura *et al.*, 1997; Solano, *et al.*, 1995) or ii) The isolation protocol could have

failed to isolate the trypanosomes from the mouthparts, but because the concentration of trypanosomes used from the pooled sample batches was very high, and that all the 10 tsetse flies were actually positive under microscope examination, this is unlikely or iii) The trypanosomes seen in the mouthparts were not actually *T. vivax*. It is reported that the sensitivity of the PCR is so high that it was able to identify a positive sample with only one trypanosome used for amplification (Katakura *et al.*, 1997). Clearly therefore, the failure to detect *T. vivax* is very likely to be due to the primers or the trypanosomes seen in the mouthparts could have been some other species. The latter explanation can be supported by the fact that all the tsetse flies dissected did not have trypanosomes in the salivary glands and therefore disqualified the presence of *T. brucei* under the microscopic examination results. However, the PCR amplification indicated that 42.9% of the samples tested were *T. brucei* positive. In fact there seems to be a high incidence of tsetse flies carrying *T. brucei* than had earlier been observed.

The finding with regards to *T. brucei* and *T. congolense* are in contrast with other documented reports that *T. vivax* is the most common trypanosome found in the vectors followed by *T. congolense* while, *T. brucei* was considered to be very rare (Woolhouse *et al.*, 1993; Bealby *et al.*, 1996). It is also documented that the most common trypanosome observed in wild mammals in the Luangwa valley is reported to be *T.*

congolense followed by *T. vivax* and least is *T. brucei* (Keymer, 1969; Dillman & Townsend, 1979). Similarly, the infection rates in goats in the same area is reported that *T. vivax* is the most prevalent trypanosomes followed by *T. congolense* and least is *T. brucei* (Bealby *et al.*, 1996). But this was observed to be a result out of the expectation to that of Keymer (1969) and Dillman & Townsend (1979) where the commonest trypanosome was *T. congolense*. It may be speculated therefore that the manifestation of trypanosomes in the mammalian host could have species suppression phenomenon. Lubinga (1996) analysing blood spots collected from cattle in Petauke district using different diagnostic techniques including PCR found that the proportions of the *T. vivax*, *T. congolense* and *T. brucei* trypanosome species affecting cattle were equal though parasitological examination had indicated more predominance of *T. congolense* over the *T. brucei* and *T. vivax*.

The results in this study in fact contradict the widely held view that *G. brevipalpis* does not carry *T. brucei*. In fact *G. brevipalpis* is a potential vector of *T. vivax*, *T. congolense* and *T. brucei*, it has clearly been proven that *T. congolense* and *T. brucei* are carried by *G. brevipalpis*. It would be more interesting to find out the favoured mammalian host on which *G. brevipalpis* feed frequently. Because *G. brevipalpis* is found near rivers, it is not only a potential threat to livestock but to humans if they ventured in their habitat and *vice-versa*

Although this study was only a preliminary investigation of trypanosomes infectivity in *G. brevipalpis*, the results obtained especially on the identification of *T. brucei* in tsetse fly and *G. brevipalpis* in particular, were very crucial to the understanding of the vector status of this species. It is important that a more comprehensive study be carried out to ascertain the actual prevalence of the *T. brucei* infections and prevalence of mixed infections in tsetse flies using PCR.

CHAPTER 7

GENERAL DISCUSSION

The study of tsetse flies in the *Fusca* group should be stepped up because the population growth rate of 4% for people living in the sub-Saharan Africa is the highest in the world and the resulting pressure on land for both arable and pastoral farming is increasing. The increase on the land pressure will lead to contact between man, domestic animals and tsetse flies which will result in out breaks of both human and animal trypanosomosis. Although land pressure in Zambia is increasing, it is far from worrying due to the re-distribution of the population that has occurred in the last 15 years which has seen increased urbanisation in the country with about 50% of the population being urban or peri-urban. Lusaka, the capital city, is the home to 20% of Zambians. This redistribution of the population has in fact lead to the spread of tsetse flies to areas which were previously settled but now are without people.

Since *G. brevipalpis* has been shown in this study to be restricted to riverine vegetation, its invasion to agricultural land should be anticipated especially in the Luangwa Valley. The introduction of the South Luangwa Area Management Unit (SLAMU) has led to reduced poaching in the GMAs leading to more tsetse fly hosts (wild animals) getting closer to

human settlements and since most settlements are along rivers, the importance of this species as a vector of trypanosomosis should increase.

The above stated situation means that while researching on *G. m. morsitans* and *G. pallidipes*, *G. brevipalpis* should also be placed in the main stream of tsetse fly research similar to that accorded with the other two species. More effort should be particularly aimed at investigating more efficient sampling systems for *G. brevipalpis* other than the present artificial refuges and electric screen fly rounds. The use of artificial refuges is restricted to the dry season while electric screen fly rounds can not be used in the flooded riverine habitat during the rainy seasons. Developing a sampling system with an all year round use can help to increase our efforts in understanding the ecology and control of *G. brevipalpis*. It is recommended therefore, that more research should be concentrated on elucidating the behaviour of this tsetse fly species when it is near baits, especially on factors which affect its trap entry. Investigations on the response of *G. brevipalpis* to natural ox-odour should be able to increase our understanding on the response of this tsetse fly species to odours.

Control of this tsetse fly species should be relatively easy due to its restricted habitat. It is likely that the use of insecticide treated artificial refuges and targets can help to effectively control *G. brevipalpis*.

While collecting data for this study over 100 blood spots were collected from fed *G. brevipalpis* to identify the sources so that preferred hosts are known and by knowing the preferred host the diurnal & feeding habits of the fly can also be known. In other countries, *G. brevipalpis* most preferred host is the hippopotamus (FAO, 1980) whose nocturnal feeding habits coincide with the times that *G. brevipalpis* is most active. These blood samples are yet to be analysed. Until the bloodspots are analysed, this aspect of the ecology of *G. brevipalpis* will remain largely unknown. The question of whether or not *G. brevipalpis* practices a sit and wait strategy or ranges in a wide foraging feeding habit can not be answered easily without having the vital data on *G. brevipalpis* preferred host in the field.

The question of catch interpretation from sampling systems has been brought out in its complexity - If you use artificial refuges in the riverine vegetation as a measure of the population composition of tsetse flies, *G. brevipalpis* would be said to be the most abundant tsetse fly species with *G. m. morsitans* and *G. pallidipes* being sparsely populated. On the other hand, epsilon traps suggest that *G. pallidipes* is the most abundant tsetse fly followed by *G. m. morsitans* with very few *G. brevipalpis*. Interpretation of tsetse fly catch composition is therefore an extremely

difficult thing to do even with the known sampling biases of the catching systems.

The age structure of tsetse flies captured by epsilon traps and artificial refuges unlike those reported elsewhere were similar. The age structure was normally distributed around the average age and the bias reported by other workers in the field (Hargrove, 1991, Vale, 1974b) especially from traps was not observed. Traps were observed to sample more females but the bias towards older females was not observed. The study also confirmed the predominance of *T. vivax* and *T. congolense* in tsetse flies on microscopic examination of the infected flies while *T. brucei* was very rare indeed. Out of over 5000 tsetse flies dissected, no tsetse fly had trypanosomes in the salivary glands but using the PCR amplification technique it was shown that about 43% of the midgut infections in *G. brevipalpis* were *T. brucei*. No further studies were carried out to find out if the 43% included mixed infections with *T. congolense*.

It was also observed that *G. brevipalpis* behaviour towards traps, fly rounds, electric screens and artificial refuges is unique to the other tsetse fly. Hence, studies on *G. pallidipes* and *G. m. morsitans* can not be extrapolated to *G. brevipalpis* or *vice-versa* and hence each of these tsetse species effectively require independent study.

REFERENCES

- Akafekwa, G. & Awan, M.A.Q.** (1975) Trypanosomiasis in Zambia. *Vetsetse Bulletin* **1**; 3-4.
- Allsop, R., Baldry, D. A. T. & Rodrigues, C.** (1972) The influence of game animals on the distribution and feeding habits of *Glossina pallidipes* in the Lambwe Valley. *Bulletin of the World Health Organisation*, **74**, 795-809.
- Allsop, R., & Coutts, H.H.** (1977) Aerial spraying trials with U.L.V. azemethiphos, crotoxyphos and Endosulphan for tsetse fly control in Botswana, 1976. *Miscellaneous reports No. 32*. Centre for Overseas Pest Research, London.
- Andrewertha, H.G. & Birch, L.C.** (1954) *The Distribution and Abundance of Animals*. Chicago, University of Chicago Press.
- Bax, S.N.** (1937) The sense of smell and sight in *Glossina swynertoni*. *Bulletin of Entomological Research*, **28**, 539-581.
- Bealby, K., Connor, R. & Rowland, J.** (1996). *Trypanosomosis in goats in the Luangwa Valley of Zambia*. ILRI 1996.
- Boyt, W.P.** (1980) *A field guide for diagnosis, treatment and prevention of African animal trypanosomiasis*. FAO, Rome.
- Brady, J & Griffiths, N.T.** (1993) Upwind flight response of tsetse flies (Diptera Glossinidae) to acetone, octenol and phenols in nature: a video study. *Bulletin of Entomological Research*, **83**; 329-333.

- Brightwell, R, Dransfield, R. D. and Kyorku, C.** (1991). Development of a low cost tsetse trap and odour baits for *G. pallidipes* and *G. longipennis* in Kenya. *Medical and Veterinary Entomology*, **5**, 153-164. ✓
- Bursell, E.** (1970) Dispersal and concentration of *glossina*. In "The African trypanosomoses" (Ed. by H.W. Mulligan). George Allen and Unwin, London. ✓
- Buxton, P.A.** (1955) *The natural history of the tsetse flies*. Memoir of the London School of Hygiene and Tropical Medicine no. 10. H.K. Lewis, London. ✓
- Bylis, M., Mbwabi, A.L. & Stevenson, P.** (1994) The feeding success of tsetse flies *Glossina pallidipes* (Diptera: Glossinidae) on oxen treated with pyrethroid pour-ons at Galana Ranch, Kenya. *Bulletin of Entomological Research*, **84**, 447-452. ✓
- Caughley, G.** (1977) *Analysis of Vertebrate populations*, pp232, Chichester, John Willey & Sons. ✓
- Challier, A.** (1965) *Amelioration de la method de determination de l'age physiologique des glossines. Etudes faites sur Glossina Palpalis palpalis Vanderplank, 1949. Bulletin de la societe de pathologie exotique*, **58**; 250-259. ✓
- Challier, A and Laveissierre, C.** (1973). Un nouveau piege pour la capture des Glossines (*Glossina: Diptera Muscidae*): description et essais sur la terrain. Cahier ORSTOM, Serie entomologie et parasitologie, **11**, 251 - 262. ✓

- Challier, A. & Turner, D.A.** (1985) Methods to calculate the survival rate in tsetse fly (*Glossina*) populations. *Annals de la societe Belge Medicine Tropicale*, **65**; 191-197. ✓
- Chorley, C. W.** (1933) Traps for tsetse flies in the "crinoline" and "ventilator" forms. *Bulletin of entomological Research*, **24**, 315-317. ✓
- Clark, L.R., Geier, P. W., Hughes, R.D. & Morris, R.F.** (1967) *The ecology of insect populations in theory and practice*. Methuen, London. ✓
- Cumming, D.H.M.** (1970) A contribution to the biology of warthog (*Phacochoerus africanus* Gmelin) in the Sengwa region of Rhodesia, Ph.D. thesis, Rhodes University. ✓
- Curtis, C.F. & Langley, P.A.** (1972). Use of Nitrogen and Chilling in production of radiation-induced sterility in the tsetse fly *Glossina morsitans*. *Ent. exp. & Appl.*, **15**, 360-376. ✓
- Dempster, J.P.** (1983) The natural control of populations of butterflies and moths. *Biological reviews*, **58**; 461-481. ✓
- Dillman, J.S.S. & Townsend, A.J.** (1979) A trypanosomosis survey of wild animals in the Luangwa Valley, Zambia. *Acta Tropica*, **36**, 349-356. ✓
- Dransfield, R.D., Chaudhury, M.F.B., Tarimo, S.A., Golder, T.K. & Brightwell, R.** (1985) Population dynamics of *Glossina pallidipes* under drought conditions at Nguruman, Kenya. *ISCTRC*, **18**; 284-292. ✓
- Du Toit, R.** (1954) Trypanosomiasis in Zululand and control of tsetse flies by chemical means. *Onderstepoort Journal of Veterinary Research*, **26**, 317-387. ✓

- FAO**, (1980) *Training manual for tsetse control personnel*, vol. **1**. ✓
- FAO**, (1992) *Training manual for tsetse control personnel*, vol. **4**. ✓
- Flint, S.** (1985) A comparison of various traps for *Glossina* spp (Glossinidae) and other Diptera. *Bulletin of Entomological Research*, **75**, 529-534. ✓
- Ford, J. & Katondo, K.M.** (1977) The distribution of tsetse flies (*Glossina* spp) in Africa 1973. Organisation of African Unity - Scientific and Technical research commission, Cook, Harmond and Kelly Ltd., London.
- Gibson, G., Packer, M.J., Steullet, P. & Brady, J.** (1991) Orientation of tsetse flies to wind, within and outside odour plumes in the field. *Physiological Entomology*, **16**, 47-57. ✓
- Glasgow, J.P.** (1963) *The distribution and abundance of tsetse*. Pergamon press, London. ✓
- Green, C.H.** (1986) Effects of colours and synthetic odours on the attraction of *Glossina pallidipes* and *G. morsitans morsitans* to traps and screens. *Physiological Entomology*, **11**, 411-421. ✓
- Green, C.H.** (1994) Bait methods for tsetse fly control. *Advances in Parasitology*, **34**, 229-291. ✓
- Green, C.H. & Flint, S.** (1986) An analysis of colour effects in the performance of the F2 trap against *Glossina pallidipes* Austen and *G. morsitans morsitans* West wood (Diptera: Glossinidae). *Bulletin of Entomological Research*, **76**, 409-418. ✓

Groenendijk, C.A. (1996) *The behaviour of tsetse in an odour plume*.
Ph.D. thesis, The University of Wageningen, The Netherlands.

GRZ reports (1996) *National strategic plan for tsetse control and rural development (1997-2001)*.

Hargrove, J.W. (1981a) Tsetse dispersal reconsidered. *Journal of Animal Ecology*, **50**, 351-373.

Hargrove, J.W. (1981b) Discrepancies between estimates of the tsetse fly population using mark recapture and removal trapping techniques. *Journal of Applied Ecology*, **18**, 737-748.

Hargrove, J.W. (1991) Ovarian ages of tsetse flies (Diptera: Glossinidae) caught from mobile and stationary baits in the presence and absence of humans. *Bulletin of Entomological Research*, **81**, 43-50.

Hargrove, J.W. (1995) Towards a general rule for estimating the stage of pregnancy in field caught tsetse flies. *Physiological Entomology*, **20**, 213-223.

Hargrove, J.W. & Parker, M.J. (1993) Nutritional states of male tsetse flies (*Glossina* spp) (Diptera: Glossinidae) caught in odour baited traps and artificial refuges models for feeding and digestion. *Bulletin of Entomological Research*, **83**, 2-46

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.** (1967) Further studies on the age and trypanosome infection rates in *Glossina pallidipes* Aust., *G. palpalis fuscipes* Newst, and *G. brevipalpis* Newst. in Uganda. *Bulletin of Entomological Research*, **???**, 459-477.
- Hassel, M.P.** (1985) Insect natural enemies as regulating factors. *Journal of Animal Ecology*, **54**, 323-334.
- Hoare, C.A.** (1972) *The trypanosomes of mammals, A zoological monograph*. Blackwell Scientific Publications, Oxford.
- Hughes, J.C.** (1957a) Olfactory stimulation of tsetse flies and blow flies. *Bulletin of Entomological Research*, **48**, 581-583.
- Hughes, J.C.** (1957b) Olfactory stimulation of *Glossina palpalis* (R.-D.) by combustion products from petrol engines. *Bulletin of Entomological Society*, **48**; 581-583.
- ILRAD** (1993) Estimating the cost of animal trypanosomosis in Africa. *ILRAD Reports*, April, 1993.
- Jackson, C.H.N.** (1941). The analysis of a tsetse population. *Annals of Eugenics, Cambridge*, **10**, 332-369.
- Jackson, C.H.N.** (1946) An artificially isolated generation of tsetse flies (Diptera). *Bulletin of Entomological Research*. **37**, 291-299
- Jackson, C.H.N.** (1949) The biology of tsetse flies. *Biological reviews*, **24**, 174-199.
- Jordan, A.M.** (1979) Trypanosomosis control and land use in Africa. *Outlook on Agriculture*, Vol. 10, No. 3.

Jordan, A.M. (1986) *Trypanosomiasis control and African rural development*, Longman, London and New York.

Jordan, A.M. (1987) Prospects for intergrated control of the African trypanosomiasis. *Proceedings of the CEC International Symposium (ISPRA)* Balkema/Rotterdam.

Jordan, A.M. (1972). Productivity of *Glossina morsitans morsitans* Westwood maintained in the Laboratory, with particular reference to the Sterile - insect release method. *Bulletin of World Health Organisation*, **46**, 33-38.

Kappmeier, K. (1997) The development of odour-baited targets for the control of *Glossina spp.* (Diptera: Glossinidae) in South Africa. M. Sc. Thesis, University of Pretoria.

Katakura, K., Lubinga, C. & Chitambo, H. (1997) Detection of *Trypanosoma congolense* and *T. brucei subspecies* in cattle in Zambia by Polymerase Chain Reaction from blood collected on filter paper. *Parasitological Research*, **83**, 241-245.

Keymer, I.F. (1969) A survey of trypanosome infections in wild ungulates in the Luangwa Valley, Zambia. *Annals of Tropical Medicine and Parasitology*, Vol. 63. No. 2.

Kiragu, J.M., Green, C.H., Stevenson, P.G. and Makumi, J.N. (1997) *Glossina brevipalpis* Newstead 1910, another *fuscus* group tsetse of concern. In "24th meeting of the International Scientific Council for

Trypanosomoses Research and Control", pp. 235-241. OAU/ISTRIC, Mozambique.

Kirchoff, F.V. & Donnelson, J.E. (1993) PCR detection of *T. cruzi*, African trypanosomes and Leishmania species. in *Diagnostic molecular microbiology. Principle and application*. Persing, D.H., Smith, T.F. Tenover, F.C. & White, T.J. (eds.) p443-455.

Kyorku, C., Brightwell, R. & Dransfield, R.D. (1990) Traps and odour baits for the tsetse fly; *Glossina longipennis* (Diptera: Glossinidae).

Bulletin of entomological Research, **80**, 405-415.

Langridge, W.P. (1960) Scent attractants for tsetse flies. In "8th meeting of the International Scientific Council for Trypanosomoses Research and Control", pp. 235-241. OAU/ISTRIC, Nairobi.

Lewis, A.E (1950) Tsetse flies carried by railway trains in Kenya Colony. *Bulletin of Entomological Research*, **40**; 511-531.

Lewis, A.E (1951) Tsetse reclamation. Tsetse reclamation report, Nairobi high commission, pp. 36-56.

Lloyd, H. M. (1935) Notes on the bionomics of *Glossina swynnertoni* Austen. *Bulletin of Entomological Research*, **26**, 439-468.

Losos, G.J & Ikede, B.O. (1972) Review of pathology of disease in domestic and laboratory animals caused by *Trypanosoma congolense*, *T. vivax*, *T. brucei*, *T. rhodensiense* and *T. gambiense*. *Veterinary Pathology (supplement)* **9**; 1-71.

- Lubinga, C.** (1996) Comparative preference of *Trypanosoma Congolense*, *T. vivax* and *T. brucei* infection in Petauke district of Zambia. M. Sc. Thesis, UNZA. ✓
- Mellanby, H.** (1937) Experimental work on reproduction in the tsetse fly *Glossina palpalis*. *Parasitology*, **31**; 131-141. ✓
- Mulla, A.F. & Rickman, L.R.** (1988) How do African game control trypanosome infections? *Parasitology Today* Vol. 4, No. 12. ✓
- Mulligan, H.W.** (ed.) (1970) *The African trypanosomiases*. George Allen and Unwin, London. ✓
- Mumba, D. & Chizyuka, H.G.B.** (1987). The tsetse and trypanosomosis situation in Zambia, (*unpublished GRZ report*) ✓
- Murray, M. & Gray, A.R.** (1984) The current situation on animal trypanosomosis in Africa. *Preventive Veterinary Medicine*, **2**; 23-30 ✓
- Nash, T.A.M.** (1969) *Africa's Bane; The tsetse fly*. Collins, London. ✓
- Neville, E.M., Kappmeier, K. Venter, G.J.** (1993) Tsetse fly Research in Zululand. in *proceedings of the ninth Entomological Congress organised by the Entomological Society of Southern Africa, Johannesburg, 28 June -1 July 1993* Pretoria. Entomological Society of South Africa. ✓
- Ormerod, J.E.** (1967) Taxonomy of the sleeping sickness trypanosomes. *Journal of Parasitology*, **53**; 824-830.

Persoons, C. J. (1966) Trapping *G. pallidipes* and *G. palpalis fuscipes* in scented traps. In "eleventh meeting of the international Scientific Council for Trypanosomoses Research and Control, Nairobi". pp. 127-132. OAU/ISTRRC, Nairobi.

Pharmacia Biotech (1996) Ready-To-Go PCR beads instructions. Pharmacia Biotech.

Phelps, R.J. & Vale, G.A. (1978) Studies on populations of *Glossina morsitans morsitans* and *G. pallidipes* (Diptera: Glossinidae) in Rhodesia. *Journal of Applied Ecology*, **15**; 743-760.

Pilson, R.D & Pilson, B. M (1967). Behaviour studies of *Glossina morsitans* Westwood in the field. *Bulletin of Entomological Research*, **6**, 227-240.

Riley, J.R., Smith, A.S., Reynolds, D.R., Edwards, A.S. Osborne, J.L., Williams, I.H., Carreck, N.L. & Poppy, G.M (1996) Tracking bees with Harmonic radar. *Nature*, **379**, 29-30.

Randolph, S.E., Rogers, D.J., (1978) Feeding cycles and flight activity in field populations of tsetse (Diptera: Glossinidae). *Bulletin of Entomological Research*, **68**; 655-671.

Randolph, S.E., Rogers, D.J., & Kiilu, J. (1991) The feeding behaviour and trappability of wild female *Glossina Pallidipes* in relation to their pregnancy cycle. *Medical and Veteerinary Entomology*, **5**, 335-350.

Rogers, D. (1977) Study of a natural population of *Glossina fuscipes fuscipes* Newstead and a model of fly movement. *Journal of Animal Ecology*, **46**, 309-330.

Rogers, D.J., Randolph, S.E. & Kuzoe, F.A.S. (1984) Local variation in the population dynamics of *Glossina palpalis palpalis* (Robineau-Desvoidy) (Diptera: Glossinidae), 1. Natural population regulation. *Bulletin of Entomological Research*, **74**: 403-423.

Rogers, D.J. & Randolph S.E. (1986) Distribution and abundance of tsetse flies (*Glossina* spp). *Journal of Animal Ecology*, **55**, 1007-1025.

RTTCP (1994) *Annual report*. RTTCP.

Saunders, D.S (1960) Ovaries of *Glossina Morsitans*. *Nature*, London, **185**; 121-122.

Saunders, D.S. (1962) Age determination for female tsetse flies and age composition of samples of *Glossina pallidipes* Austen, *G. palpalis fuscipes* Newstead and *G. brevipalpis* Newstead. *Bulletin of Entomological Research*, **53**; 579-595.

Saunders, D.S. (1964) The effect of site and sampling method on the size and composition of catches of tsetse flies (*Glossina*) and tabanidae (Diptera). *Bulletin of Entomological Research*, **55**, 483-497.

SEMG (1993) Environmental monitoring of tsetse control operations in Zambia and Zimbabwe: Impact of aerial spraying and odour baited targets on Eco-systems (1987 - 1990)

Solano, P., Argiro, L., Reifenberg, J.M., Yao Yao, & Duvallet, G.

(1995) Field application of the Polymerase Chain Reaction (PCR) to the detection and Characterisation of trypanosomes in *Glossina longipalpis* (Diptera: Glossinidae) in Cote d'Ivoire. *Molecular Ecology*, **4**, 781-785.

Soulsby, E.L.J. (1986) *Helminths, Arthropods and Protozoa of Domesticated Animals*. Bailliere Tindall, London.

Southwood, T.R.E. (1980) *Ecological methods*. Methuen & Co. Ltd., London.

Synnerton, C.F.M. (1936) The tsetse flies of East Africa. A first study of their ecology with a view to their control. *Transaction of the Royal Society of London* Vol. 84.

Turner, D.A. (1987) Population Ecology of *Glossina pallidipes* Austen (Diptera: Glossinidae) in the Lambwe Valley, Kenya. 1. Feeding behaviour and activity patterns. *Bulletin of Entomological Research*, **77**; 317-333.

Torr, S.J. (1989) The host oriented behaviour of tsetse flies (*Glossina*): the interaction of visual and olfactory stimuli. *Physiological Entomology*, **14**, 325-340.

Torr, S.J. (1990). Dose response of tsetse flies (*Glossina*) to carbon dioxide, acetone and octenol in the field. *Physiological Entomology*, **15**, 93-103.

Torr, S.J. (1994) A possibility of using artificial refuges to control tsetse flies. *RTTCP Reports*, April, 1994.

- Urquhart, G.M., Armour, J., Duncan, J.L., Dunn, A.M. & Jennings, F.W.** (1996) *Veterinary Parasitology* Blackwell Science Ltd. Oxford.
- Vale, G. A.** (1971). Artificial refuges for tsetse flies (*Glossina* spp). *Bulletin of Entomological Research*, **61**, 331-350.
- Vale, G. A.** (1974a) New field methods for studying the responses of tsetse flies (Diptera: Glossinidae) to hosts. *Bulletin of Entomology Research*, **64**, 199-208.
- Vale, G. A.** (1974b). The responses of tsetse flies (Diptera: Glossinidae) to mobile and stationary baits. *Bulletin of Entomological Research*, **64**, 545-588.
- Vale, G.A. & Cumming, D.H.M.** (1976) The effects of selective elimination of hosts on tsetse flies (*G. m. morsitans* Westwood (Diptera: Glossinidae)). *Bulletin of Entomological Research*, **66**, 713-729.
- Vale, G.A. & Hargrove, J.W.** (1979) AS method for studying the efficiency of traps of tsetse. *Bulletin of Entomological Research*, **69**, 183-193.
- Vale, G.A** (1982) The trap-oriented behaviour of tsetse flies (Glossinidae) and other Diptera. *Bulletin of Entomological Research*, **72**, 71-93.
- Vale, G.A., Hursey, B.S., Hargrove, J.W., Torr, S.J & Allsopp, R.** (1984) The use of small plots to study population of tsetse (Diptera: Glossinidae): difficulties association with population dispersal. *Insect science and its Applications* **5** (5), 403-410.

- Vale, G.A., Bursell, E. and Hargrove, J.W.** (1985) Catching out the tsetse fly. *Parasitology Today*, **Vol. 1 number 4**, 106-110.
- Vale, G. A. & Hall, D.R.** (1985) The role of 1-octen-3-ol, acetone, and carbon dioxide in the attraction of tsetse flies, *Glossina* spp. (Diptera: Glossinidae), to ox odour. *Bulletin of Entomological Research*, **75**, 209-217.
- Vale, G.A., Hargrove, J.W., Cockbill, G.F. & Phelps, R.J.** (1986) Field trials of baits to control populations of *Glossina morsitans morsitans* Westwood and *Glossina Pallidipes* Austen (Diptera: Glossinidae). *Bulletin of Entomological Research*, **76**, 179-193.
- Vale, G.A., Lovemore, D.F., Flint, S., & Cockbill, G.F.** (1988a) Odour baited targets to control tsetse flies, *Glossina* spp. (Diptera: Glossinidae), in Zimbabwe. *Bulletin of Entomological Research*, **78**, 31-49.
- Vale, G. A., Hall, D.R. & Gough, A.J.E.** (1988b) The olfactory responses of tsetse flies, *Glossina* spp. (Diptera: Glossinidae), to phenols and urine in the field. *Bulletin of Entomological Research*, **78**, 293-300.
- Vale, G. A.** (1993) Development of baits for tsetse flies (Diptera: Glossinidae) in Zimbabwe. *Journal of Medical Entomology*, **30**; No 5, 831-842.
- Vale, G.A.** (1994) *The philosophical problems of tsetse distribution.* RTTCP Miscellaneous Reports.

- Vale, G.A.** (1998) Responses of tsetse flies (Diptera: Glossinidae) to vegetation in Zimbabwe: implications for population distribution and bait siting. *Bulletin of Entomological Research*, **88**, S7-S59.
- Vanderplank, F.L.** (1944) Studies of the behaviour of the tsetse fly *Glossina pallidipes* in the field: The attractiveness of various baits. *Journal of Animal Ecology*, **13**; 39-48.
- Van Sickle, J. & Phelps, R.J.** (1988) Age distribution and reproductive status of declining and stationary populations of *Glossina pallidipes* Austen (Diptera: Glossinidae) in Zimbabwe. *Bulletin of Entomological Research*, **78**, 51-61.
- Williams, B., Dransfield, R., Brightwell, R., & Rogers, D.** (1993) Where are we now? Trypanosomosis. *Health Policy and Planning*, **81**, (1) Vol. 8. No. 1.
- Williamson, D.L., Dame, D.A., Gates, D.B., Cobb, P.E., Bakuli, B. & Warner, P.V.** (1983) Intergration of insect sterility and insecticides for control of *Glossina morsitans morsitans* Westwood (Diptera: Glossinidae) in Tanzania. V. The impact of the sequential releases of sterilised tsetse flies. *Bulletin of Entomological Research*, **73**, 391-404.
- Williemse, L.** (1991) A trial of odour baited targets to control the tsetse fly *Glossina morsitans centralis* (Diptera: Glossinidae) in West Zambia. *Bulletin of entomological Research* **81**, 351-357.

Woolhouse, M.E.J., Hargrove, J.W. & McNamara, J.J. (1993)

Trypanosome infections of the tsetse fly *Glossina pallidipes* in the
Luangwa Valley, Zambia. *International Journal of Parasitology*, **24**, 987-
993.