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Tsetse control, diagnosis and chemotherapy using nuclear techniques

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and the
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TSETSE CONTROL, DIAGNOSIS AND CHEMOTHERAPY
USING NUCLEAR TECHNIQUES

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FOREWORD

The presence of tsetse flies, vectors of the potentially fatal human and livestock disease, trypanosomiasis, continues to place a major constraint on agricultural development and severely limits the productive use of land over most of sub-Saharan Africa. In view of the magnitude of the problem, great efforts have been directed in the past towards controlling tsetse flies and the disease by a variety of indirect and direct methods against the vectors, as well as refining diagnostic methods, chemoprophylaxis and chemotherapy.

The IAEA, through its Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, is assuming responsibility for research and development of nuclear techniques, including biotechnology, for tsetse and trypanosomiasis control. Within the policy framework of the FAO Action Programme for the Control of African Animal Trypanosomiasis and Related Development, the Joint FAO/IAEA Division's role is to contribute new techniques, to test these under field conditions and to recommend them for integration in selected geographical areas where tsetse and trypanosomiasis control is technically feasible and economically advantageous.

The main focus of the present Seminar on African Animal Trypanosomiasis: Tsetse Control, Diagnosis and Chemotherapy Using Nuclear Techniques, jointly organized by the IAEA and FAO, was on recent advancements in the use of nuclear techniques for the control of tsetse-transmitted trypanosomiasis. Equally important objectives were to provide a forum for maximum interdisciplinary discussion on available control methods, and to stimulate multidisciplinary research approaches, together with greater co-operation and practical collaboration between the international organizations/research centres and specialized national institutes in meeting the difficult challenges posed by animal trypanosomiasis.

These proceedings contain the extended synopses of all papers presented at the Seminar and the full text of 16 selected papers dealing with disease, diagnosis, chemotherapy, vector biology, ecology and control. With the exception of slight formal changes during retyping and incorporation of tables and figures into the text, no major revision of the submitted papers was done.

The help of all who took part in organizing the Seminar is highly appreciated.

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CONTENTS

Introduction 9

Opening Address 11
H.L. Norton (FAO Representative)

Summary of Sessions 13

PART I. DIAGNOSIS OF ANIMAL TRYPANOSOMIASIS

The development and application of antigen-detection enzyme immonoassays (antigen-ELISA) for diagnosis and control of African trypanosomiasis 21
W.M. Nantulya, K.J. Lindqvist, R.A. Masake

Application of antigen-ELISA for the diagnosis of Surra in selected camel herds in Kenya ... 25
W. Olaho-Mukani, W.K. Munyua, J.K. Omuse, A.R. Njogu, M.W. Mutugi

Validation of the ELISA technique for diagnosis of trypanosomiasis in cattle in Uganda *(Summary)* 31
N.M. Okuna

Current status of in vitro assays for identifying drug-resistant trypanosomes *(Summary)* 33
R. Kaminsky

Pharmacokinetics of diminazene aceturate (Berenil^{R}), homidium bromide (Ethidium^{R}) and isometamidium chloride (Samorin^{R}) after intravenous application in Boran steers *(Summary)* 35
R.D. Kratzer, A. Ismail, J. Omukuba, V. Cagnolati

Investigations of intramuscularly induced diminazene aceturate (Berenil^{R}) plasma levels in cattle under high tsetse and trypanosome challenge in the field *(Summary)* 37
R.D. Kratzer, K.F. Loehr, S. Maloo, S. Muenstermann, A. Ismail, J. Omukuba, G. Murilla

A comparison of intramuscularly induced plasma levels of diminazene aceturate (Berenil^{R}), homidium bromide (Ethidium^{R}) and isometamidium chloride (Samorin^{R}) in infected and uninfected cattle *(Summary)* 39
R.D. Kratzer, P.K. Turkson, A. Ismail, J. Omukuba, V. Cagnolati

Sorbent extraction and high performance liquid chromatography (HPLC) of homidium bromide and isometamidium chloride in bovine plasma *(Summary)* 41
R.D. Kratzer, W.M. Karanja, G. Murilla

PART II. CHEMOTHERAPY OF ANIMAL TRYPANOSOMIASIS

Solid phase extraction and reversed-phase high performance liquid chromatography of homidium in animal tissues 45
G.A. Murilla, R.E. Mdachi, R.D. Kratzer, W.M. Karanja

Metabolite studies of isometamidium in cattle 53
R.E. Mdachi, G.A. Murilla, R.D. Kratzer

Preliminary studies on the uptake and efflux of radiolabelled drugs by susceptible and drug-resistant *Trypanosoma brucei brucei (Summary)* 59
R. Kaminsky, E. Zweygarth, R.D. Kratzer

Use of radiolabelled trypanocides for sensitivity screening *(Summary)* 61
Hailemariam Lemecha

PART III. TSETSE FLY BIOLOGY, ECOLOGY, VECTORIAL CAPACITY

Fat and haematin contents of male tsetse flies *Glossina pallidipes* and *G. m. morsitans* (Diptera: Glossinidae) caught in odour-baited traps and artificial refuges in Zimbabwe 65
J.W.Hargrove, M.J. Packer

Study of the vectorial capacity of *Glossina palpalis palpalis* related to its digestive physiology and rearing conditions 91
J. Van den Abbeele, W. Decleir

Biosynthesis of lipids in female tsetse flies following matings with radiation-sterilized or normal males 105
E.N. Lambremont, M. Taher

Comparative study on the susceptibility of different *Glossina* species to *Trypanosoma vivax, T. congolense* or *T. b. brucei (Summary)* 119
S.K. Moloo

Vectorial capacity of *Glossina palpalis gambiensis* after combined radiation and low temperature treatment during the late pupal stage *(Summary)* 123
A. Djiteye, B. Bauer, A. Van der Vloedt, U. Feldmann, M.J.B. Vreysen

Effect of irradiation on tsetse flies *(Summary)* 127
D.H. Molyneux, J.K. Stiles

Study of tsetse–host immunity relationships with respect to mass rearing and tsetse eradication 129
T. Soldán, V. Maťha, P. Kopáček, P. Volf, F. Weyda

PART IV. TSETSE FLY CONTROL, TSETSE GENETICS AND TSETSE REARING

The durability of pyriproxyfen, a juvenile hormone mimic, for insect control 143
P.A. Langley, B. Mauchamp, C. Royer, H. Oouchi

Generic studies on *Glossina morsitans* and *Glossina palpalis* related to genetic control 151
R.H. Gooding

Tsetse fly mass rearing: Colony management, deployment of sterile flies, related research and development 167
U. Feldmann, D. Luger, H. Barnor, L. Dengwat, B. Ajagbonna, M.J.B. Vreysen, A. Van der Vloedt

PART V. VECTOR CONTROL: TRAINING, USE OF TARGETS AND COUNTRY PROGRAMMES

A study of factors affecting the persistence of deltamethrin applied to cotton fabric for tsetse fly control 183
M. Hussain, H. Perschke

Experience with odour baited insecticide impregnated targets for control of tsetse flies in Kenya *(Summary)* 191
E.A. Opiyo, J.K. Omuse

Field testing of improved insecticide formulation for control of tsetse using baited targets *(Summary)* 193
E.A. Opiyo, J.K. Omuse, M. Hussain, J. Kiragu

Development of controlled release formulations of pesticides using nuclear techniques for the control of tsetse flies *(Summary)* 195
H.T. Matechi, D.N. Maeda, P.S.M. Macha, S.R. Mbise, M. Sikay

Simple creation of controlled release formulations of insecticides by a polymeric spray tank additive 197
G.G. Allan, R.G. Allan, J.P. Carroll, A.N. Neogi
Tsetse and trypanosomiasis control in Zimbabwe *(Summary)* 205
V. Chadenga
Integrated tsetse management in southwestern Ethiopia *(Summary)* 207
G. Tikubet
Interim strategies to control animal trypanosomiasis in two selected villages along the White Volta river in the onchocerciasis free zone of northern Ghana *(Summary)* 209
C.K. Doku, C.I. Mahama
Trypanosomiasis control and eradication of *Glossina austeni* from Zanzibar using an integrated approach *(Summary)* 211
K.M. Biwi
Control of *Glossina austeni* and cattle trypanosomiasis in Unjuga island by deltamethrin pour-on application to livestock and with stationary targets in cattle-free zones 213
D.W. Höreth-Böntgen
Laboratory and field observations in relation to the release of sterile *Glossina austeni* on Unjuga (Zanzibar) island 219
M.J.B. Vreysen, F. Mramba, I. Khamis
Tsetse, trypanosomiasis and cattle raising in southern Anambra State, Nigeria *(Summary)* 231
G.O.C. Ekejindu, U.C. Nnamani, O.C. Ogamba
Comparison of baited and unbaited NGU and biconical traps for *Glossina pallidipes* and *G. longipennis* and the contribution of odour combinations in Nguruman, Kajiado District, Kenya 233
F.P. Oloo
Activities of the Tsetse Control Unit, Botswana *(Summary)* 243
T.K. Phillemon-Motsu
Animal trypanosomiasis control in Uganda *(Summary)* 245
E.B. Gamurorwa
Tsetse and trypanosomiasis control for the rural development of Buvuma Island, Lake Victoria, Uganda *(Summary)* 247
L.M. Ogwal, T.N. Kangwagye, L. Semakula, C. Ndyabahika, P. Esiru
Tsetse and trypanosomiasis at the Ngulia Rhino Sanctuary *(Summary)* 249
S. Mihok, E. Munyoki, J.F. Jonyo, D. Rottcher, R.A. Brett, P.O. Majiwa
An evaluation of the suppression of *Glossina pallidipes* by the odour-baited insecticide-coated targets in the Lambwe Valley, Kenya *(Summary)* 251
M.M. Mohamed-Ahmed, L.H. Otieno, J. Muchiri

List of Participants 253

INTRODUCTION

The seminar was held at the Kenya Trypanosomiasis Research Institute (KETRI) Conference Centre at Muguga/Nairobi, Kenya, and lasted 5 days, including half a day for optional visits to other laboratories (ILRAD, ICIPE) engaged in research on tsetse and trypanosomiasis, and one day devoted to visiting the Nguruman field site (Maasailand, south of Nairobi) where long-range studies on cattle herds under trypanosomiasis challenge and on the ecology and control of the tsetse fly are in progress.

In order to enhance multidisciplinary interaction between entomologists, tsetse ecologists, parasitologists and veterinarians from and outside of Africa, Research Co-ordination Meetings (RCMs) of two related Co-ordinated Research Programmes (CRPs) were held in conjunction with the seminar. One is the CRP on "Development of Practices for Area-wide Tsetse Eradication or Control with Emphasis on the SIT" conducted by the Joint FAO/IAEA Division's Insect and Pest Control Section. The second one is the CRP on "Development of Controlled-release Formulations of Pesticides Using Nuclear Techniques" conducted by the Agrochemicals and Residues Section. Therefore, progress reports prepared by the scientists participating in these CRPs were integral parts of the Seminar programme. Moreover, the fact that the Joint Division's Animal Production and Health Section is also co-ordinating the Netherlands Government funded FAO/IAEA/ILRAD network activities on ELISA validation, and that KETRI scientists at Muguga are conducting research on the "Fate of Trypanocidal Drugs in Cattle", made it possible to incorporate in the provisional seminar programme sections dealing with diagnosis and chemotherapy of animal trypanosomiasis. A total of 106 registered participants and observers attended, including 17 nominated by various African countries (Botswana, Burkina Faso, Burundi, Congo, Côte d'Ivoire, Ethiopia, Guinea, Mali, Rwanda, Sierra Leone, Sudan, Uganda (2), United Republic of Tanzania (2), Zambia and Zimbabwe.

IAEA Research Contract/Agreement holders present came from Belgium, Canada, the Czech and Slovak Federal Republic, Ethiopia, Ghana, Kenya, Uganda, United Kingdom, United Republic of Tanzania, USA and Zimbabwe. Scientists and technologists from KETRI, ILRAD, ICIPE and the University of Nairobi also contributed substantially to specific technical sessions.

The FAO was represented by Mr. H.L. Norton (Representative in Kenya), Mr. K. Gyening (Regional Office for Africa, Accra), Mr. B.S. Hursey (Senior Trypanosomiasis Officer, AGAH, Rome), Mr. D. Höreth-Böntgen (URT/86/022, Zanzibar) and Mr. M. Vreysen (FAO/IAEA APO, URT/5/007, Zanzibar).

The Joint FAO/IAEA Division was represented by its technical officers M. Hussain (Agrochemicals Unit), C.J. Ooijen (Animal Production and Health Section), U. Feldmann (Entomology Unit) and A. Van der Vloedt (Insect and Pest Control Section).

During the opening session of the seminar, Mr. H.L. Norton, newly appointed FAO Representative in Kenya, stated that the tsetse fly is arguably one of the most serious constraints imposed on development towards self-sufficiency in food production in many African countries. On the other hand, he forcefully stated that tsetse/trypanosomiasis control cannot stand alone but must be considered as only one aspect within a package of activities identified as necessary for the attainment of sustainable agricultural

production (p. 11). After further introductory comments by Mr. J.K. Omuse, Director, KETRI, on the tsetse/trypanosomiasis problem in Kenya and on KETRI's mandate, the seminar was officially opened by Mrs. Rispan Oduwo, Representative of the Kenyan Ministry of Research, Science and Technology, who, in her opening address, commended the FAO and IAEA for the decision to hold the seminar in Kenya and for the support given to national institutes.

The local organizing committee was headed by Dr. (Mrs.) Mary Otsyula.

The scientific sessions were preceded by a presentation by Mr. B.S. Hursey (FAO, Rome), in which the FAO Programme for the Control of African Animal Trypanosomiasis and Related Development was outlined, with due consideration to developments and achievements of the last 10 years, but also taking into consideration some of the constraints (e.g. changes in national priority given to control/eradication of tsetse, controversies regarding expected benefits, lack of adequate funding, needs for further training in proper implementation of available control technologies, etc.).

OPENING ADDRESS

H.L. Norton
FAO Representative in Nairobi, Kenya

I am pleased to be here this morning at the Kenya Trypanosomiasis Research Centre, to make this opening address at this Seminar on Animal Trypanosomiasis: Tsetse Control, Diagnosis and Chemotherapy, Using Nuclear Techniques, and the work that is being done to find ways to control and eradicate the tsetse fly, an insect that has affected both animal and human life, and has seriously affected the economic and social life of vast regions of Africa.

However, please allow me to digress for a few moments to extend the greetings and best wishes for a successful seminar from Director-General, Dr. Edouard Saouma and staff in FAO Headquarters and to note that I have recently arrived in Kenya from the Sudan, and am taking this and other opportunities to be involved in the making faucets of FAO work and activities.

I am proud to be able to participate in the opening of this seminar which is jointly sponsored by IAEA and FAO, with collaboration of KETRI, Kenyan and International Organizations, and countries from around the world that are working in this very important work. In keeping with the theme of this seminar, I feel I should reemphasize that the presence of the tsetse fly over most of sub-Saharan Africa continues to place a major contraint on agricultural and human development through the transmission of animal trypanosomiasis and to a lesser degree human sleeping sickness. This situation has persisted throughout the history of the continent but is becoming of increasing concern as human populations expand and the demand for land to increase food production becomes more imperative. The tsetse fly is arguably one of the most serious contraints imposed on development towards self-sufficiency in food production in many African countries.

The eradication and control of this insect has involved a great deal of controversy since the inception of these activities in the early 1920's. Much of this has been evoked by the methods employed, which in the early days included the large-scale destruction of the tsetse habitat and wild animal hosts, and more recently the extensive treatment of infested areas with chemical insecticides. All of which, although effective, may adversely effect the ecology of fragile environments. Fears have also been voiced that the removal of tsetse from wildlife reserves may promote and accelerate the uncontrolled settlement of such areas. Such fears do have some substance and are not based on groundless emotion. It is for this reason that those working in the field of tsetse and trypanosomiasis control, have in the last decade or so developed new techniques, such as odour attractive devices, the Sterile Insect Technique and insecticide treatment of livestock, which are not only environmentally acceptable but are also potentially capable to be implemented on a self-help basis.

It is, therefore, evident that tsetse/trypanosomiasis control cannot stand alone but must be considered as only one aspect within a package of activities necessary for the attainment of sustainable agricultural production. The exception to this is, of course, the alleviation of an emergency disease situation within settled areas where land use practices are already established.

In recognition of the above and the need for coordination both within and between infested countries, FAO in 1979 established the Commission for African Animal Trypanosomiasis with the support of two Expert Panels to advise on

Ecological/Technical and Related Development aspects of the Programme. These bodies are further strengthened through FAO support to national activities, coordination with donors, research funding to development aspects and regular programme support to initiating actions through the Technical Cooperation Programme. Training is also recognized as a vital activity and receives priority consideration within the limits of available funding.

The technical means to achieve the programme objectives are available, although there is scope for improvement in the efficiency of the various techniques employed. These include trypanosomiasis diagnostics, chemoprophylaxis and chemotherapy. For tsetse control, selective ground spraying, aerial spraying, the use of artificial odour attractive devices and the insecticide treatment of livestock have all produced significant results either in the control or eradication of the disease. It is now essential that these techniques be employed as part of development schemes towards improved and sustainable agricultural production.

It is this guarantee of sustainability that has so far proved elusive, mainly due to the lack of adequate funding to reach the long-term objective. This is because national authorities and donor agencies still need to be convinced of the technical and economic justification for a considerable long-term investment and it is, sadly, in this area that the information required to convince them remains largely unavailable. In order to develop a consolidated programme of action, certain facts must be known, for example, the accurate tsetse distribution, the species present, the economic and social effects of the disease on local livestock and communities, the recurring cost to Governments, the actions taken, their effectiveness and, most importantly, the national priority given to control and/or eradication and the expected benefits.

The collection and collation of this information requires the close collaboration of many agencies within national governments and it is for this reason that FAO, through the Commission, has urged Member Nations to seriously consider the formation of national coordinating committees. There is no doubt that much of the information required to justify and formulate proposals for long-term action is available but remains unseen, by those of direct concern, in many offices and archives. It is up to you, professional technical officers, to bring this information to light and, where the justification exists, bring to the attention of policy makers and administrators, the need for a concerted and determined effort towards the control of tsetse and trypanosomiasis.

I am sure that during the coming week you will, through mutual discussion and exchange, make a thorough analysis of the current status on technical aspects of tsetse and trypanosomiasis control. I urge you not to forget that the much wider issues outlined above, are of equal importance.

In closing, may I also remind you that FAO, through the Programme for the Control of African Animal Trypanosomiasis and Related Development and as a servant of its Member Nations, is ready to assist through the provision of technical expertise, the identification of projects and proposals, the seeking of funding support and last but not least, coordination within the Programme.

Thank you for this opportunity to convey the interest, thoughts and involvement of FAO in the search to find answers and solutions to control the tsetse fly and trypanosomiasis.

SUMMARY OF SESSIONS

Summary of Diagnosis Session

(Moderator/Rapporteur - C.J. Ooijen)

The presentations in this session covered the development and application of antigen-detection enzyme immunoassays (antigen-ELISA) for the diagnosis and control of animal trypanosomiasis. Prior to these presentations, an overview was given of the Netherlands Government funded FAO/IAEA Co-ordinated Research Programme on improving the diagnosis and control of trypanosomiasis in African livestock using ELISA methods.

(i) In close collaboration with ILRAD and the CTVM, the Animal Production and Health Section of the Joint FAO/IAEA Division has developed ELISA Kits for the diagnosis of bovine (ILRAD) and camel (CTVM) trypanosomiasis. The assay detects species-specific trypanosome antigens, thus providing direct evidence of current infections. Through a Co-ordinated Research Programme, a network was established in 1988 involving 12 African research institutes. After the successful introduction of the assay at these institutes, work has since been carried out to validate the diagnostic reagents. These validation results will be presented at the programme's third RCM in Côte d'Ivoire, May 1991.

(ii) Several currently used standard laboratory methods for the diagnosis of animal trypanosomiasis are either not sensitive enough or will only provide a presumptive diagnosis by detecting anti-trypanosome antibodies. Detection of trypanosome species-specific antigens released in the circulation of the infected host following immune response, would, however, provide evidence of a current infection. A trypanosome species-specific antigen-detection ELISA (bovine) using monoclonal antibodies was developed at ILRAD and subsequent validation studies demonstrated a very high sensitivity and specificity. Some preliminary results of the assay validation in Uganda indicated the increased sensitivity of the test in comparison with the Phase Contrast Buffy Coat technique and mouse inoculation. Additional work still needs to be carried out to verify the obtained ELISA results.

The presentations clearly indicated the main advantages of the antigen-detection assay, i.e. improved sensitivity and specificity as compared to currently used standard laboratory diagnostic techniques. However, further studies are still required to complete the validation of the assay at different locations in Africa, prior to its general routine application in national tsetse and trypanosomiasis control programmes. Comments in the discussions further identified the necessity to investigate the practical implications of an expected higher trypanosomiasis prevalence rate in individual countries using the more sensitive ELISA.

Summary of Chemotherapy Sessions

(Moderators/Rapporteurs - R. Kaminsky, G. Murilla, R. Kratzer)

Reports in the two chemotherapy sessions covered two major areas: the use of radiolabelled trypanocidal drugs for pharmacokinetic studies, and the application of *in vitro* techniques for assessment of drug sensitivities in trypanosomes.

(i) Differences in the pharmacokinetic characteristics of three tested drugs were demonstrated. Isometamidium chloride (Samorin^{R}) had significantly higher plasma levels after i.v. application compared to i.m. application. In contrast, only minor differences occurred in plasma levels of diminazene aceturate (Berenil^{R}) and homodium bromide (Ethidium^{R}). No differences were detected in plasma levels of diminazene aceturate in cattle under high fly challenge compared to cattle not exposed to tsetse and trypanosome infections. Also, only minor differences occurred in plasma levels of diminazene aceturate, isometamidium chloride and homidium bromide between infected and uninfected cattle. In a preliminary study, metabolites of ^{14}C-isometamidium chloride, which corresponded to homidium and benzamide were detected in liver, kidney, bile and urine samples. Two presentations dealt with the development of a cold technique to detect homidium bromide and isometamidium chloride in bovine plasma or tissues.

(ii) In an overview, the existing in vitro methods to test for drug resistance in trypanosomes were presented. Most assays use either H-hypoxanthine incorporation or growth inhibition as parameters to distinguish between drug-resistant and drug-sensitive trypanosomes. Assays for assessing diminazene aceturate, isometamidium chloride or suramin resistance in Trypanosoma brucei brucei are established. However, such assays are at the research stage regarding T. congolense and T. vivax. It was demonstrated in a preliminary study that the uptake of radiolabelled diminazene aceturate in vitro was different in drug-resistant and drug-sensitive trypanosomes, but no difference occurred when isometamidium chloride was used.

The presentations and comments in the discussions emphasized the advantages and need for further studies with radiolabelled trypanocides (i) for pharmacokinetic studies, e.g. on prophylactic activities of the drugs, residues in animal tissues and on the appearance of metabolites, and (ii) for use in in vitro studies to elucidate more characteristics of the mechanisms of drug resistance in trypanosomes.

Summary of Session on Tsetse Biology, Ecology and Vectorial Capacity

(Moderators/Rapporteurs - D.H. Molyneux and A. Van der Vloedt)

An introductory talk was given on the objectives of the Co-ordinated Research Programme on "Development of Practices for Area-wide Tsetse Eradication or Control with Emphasis on the SIT". Contractual involvements of the IAEA were explained in relation to the interactive links of contract and agreement holders.

The session encompassed studies on tsetse populations, tsetse physiology in relation to infection and irradiation, and vectorial capacity of colonised flies.

The need to study the inter-larval period, fly behaviour and feeding intervals of various Glossina species remains an essential prerequisite for all aspects of control activities. Research has involved the analysis and interpretation of fat and haematin contents of tsetse as parameters of the hunger state. Such studies have been undertaken using both traps and artificial refuges in attempts to evaluate the duration of the feeding cycle and define the availability of flies to trapping and attractive devices at different stages of the cycle.

The interrelationships between Glossina digestive enzymes and trypanosomes is a topic which has been little studied. Recent investigations have highlighted the role of tsetse midgut lectins and agglutinins in the determination of fly susceptibility to trypanosomes. Detailed studies on trypsin/trypsin-like enzyme in the midgut of G. p. palpalis have been undertaken, involving the pattern of activity, isolation of the enzyme and further studies on the interaction between digestive enzymes and parasite establishment. From available information it is also clear that the cycle of enzyme production is linked to engorgement of flies, that different Glossina species/subspecies have different levels of enzyme activity, and that irradiation reduces enzyme production.

Radio-labelled lipid precursor molecules have been used to investigate the biosynthesis of lipids in female G. p. palpalis following matings with radiation-sterilized or normal males. Synthesis of total lipids, total phospholipids and neutral lipids was normal as monitored by labelled ^{14}C-1,2 acetate incorporation, but synthesis of the 1,2-diacylglycerols was distinctly depressed in the uterine glands of females mated to irradiated males.

Studies on the vectorial capacity of colonised Glossina species of different feeding regimen (in vivo/in vitro feeding), maintained under a variety of incubation conditions and given a radiation treatment in the pupal stage were also reported. It is known that considerable variation in the laboratory results can be obtained, even when the same trypanosome stocks are used. What obviously remains valid is that: (i) T. vivax infection rates tend to be higher than T. congolense and T. brucei type of infections, (ii) salivary gland infection rates of the latter species are low and (iii) T. congolense is not transmitted well by palpalis group species. It is also clear that even under the most controlled conditions, Glossina infection rate studies can provide limited data relevant to field situations without a knowledge of the mechanisms which control fly susceptibility and parasite maturation. Studies in this area were reported which involved attempts at determining differences in specificities for carbohydrate residues (on the surface of human erythrocytes) of haemagglutinins in susceptible and refractory lines of G. m. morsitans and in palpalis subspecies. Other differences in midgut agglutinins and lysins (lectin-like molecule) between palpalis subspecies have been observed. G. p. palpalis appears to have a lytic molecule located in the posterior midgut which is absent from G. p. gambiense. Moreover, differences in the biochemical profiles of the salivary glands correlate with the low, if not total lack of, susceptibility of G. p. palpalis (from an in vitro fed stock colony) to T. brucei.

The effects of irradiation on tsetse midgut structures were also discussed. Major damage occurs to midgut and peritrophic membrane when flies are treated at doses comparable to those used in SIT programmes. However, the damage is repaired after about 10 days and does not appear to significantly effect the flies' longivity and their ability to establish and develop mature infections.

Considering the results presented and discussions during this session, it is clear that:

(i) There is a need to continue evaluating the vectorial capacity of tsetse flies in relation to their ability to transmit pathogenic trypanosomes, but this work should be done under strictly controlled conditions. Laboratory studies, using well-defined Glossina populations and trypanosome stocks, should be orientated towards elucidating the mechanisms which determine fly susceptibility and parasite maturation. In particular, it is recommended that research

be concentrated on characterisation of molecules known to have activity against trypanosomes. Moreover, research for a better understanding of the genetics of fly susceptibility should also be conducted.

(ii) Field studies should concentrate on defining the importance of particular tsetse fly species as vectors of trypanosomiasis. Planning and implementation of control activities should always be done based on well-documented importance of particular tsetse species as vectors and the cost-effectiveness of their control with due consideration to livestock production priorities and land use potential. Such pre-control feasibility studies should employ appropriate methodologies (molecular and species-specific DNA probes) and be coordinated to provide data on the age structure of the Glossina populations and other qualitative (e.g. presence of endosymbionts, preferred host) and quantitative characteristics of epidemiological importance.

(iii) Physiological and ecological studies on Glossina should continue in particular when they can provide information pertinent to control operations.

Summary of Session on Tsetse Control (Part 1), Genetics and Tsetse Rearing

(Moderators/Rapporteurs - P. Langley and R.H. Gooding)

Papers in this session dealt with the prospects for controlling tsetse populations without using conventional insecticides. These included the role of tsetse host-immunity relationships in the development of control mechanisms, chemical sterilization of natural populations of tsetse using a juvenile hormone mimic, inter-subspecific hybrid sterility, the use of "satyrs" (males of one species mating with females of another species) and the SIT.

Tsetse host-immunity relationships have been studied with a view to identifying immunologically active molecules which can be maintained in the tsetse host and which are lethal to the fly. This approach would be especially applicable in areas where cattle or other domestic animals constitute the major hosts of tsetse.

Juvenile hormone mimics as substitutes for highly toxic or mutagenic chemosterilants have much to commend them. Pyriproxyfen is particularly potent and is very stable. Although it is excreted by the adult female Glossina and may be metabolised slowly, it appears not to be metabolised once it reaches the larva in tsetse (or the egg in Rhodnius). It is also stable on netting surfaces although it seems to be physically removed over a period of several months, probably due to weathering. Pyriproxyfen has very low mammalian toxicity and since it operates through an entirely different metabolic pathway than conventional insecticides, its use alongside such insecticides is recommended in order to reduce the chances of genetically based resistance developing in the target insect. The efficacy of pyriproxyfen is further enhanced (as demonstrated during tests with ^{14}C-labelled compound) by the prospect of sterilizing doses being passed on by contaminated males to adult females during mating.

Although inter-subspecific hybrid sterility has been considered in the past as a means of controlling one subspecies by the introduction of another into the same area, there are some unresolved potential problems involved in implementation of such a technique. More particularly, satyrs must (i) be competitive in nature with males of the target species, in mating with females

of the target species, (ii) be as effective in inducing monogamy and (iii) have sperm that are fully competitive with those of the target species males, if females of the target species mate more than once. As reported, the use of "satyrs" may be possible among palpalis group flies (palpalis/gambiense model) where sperm appears to be competitive with that of the conspecific males. However, this appears not to be true of morsitans group flies where conspecific sperm is utilised preferentially by females.

Other prospects for genetic manipulation are seen in those cases (fuscipes/palpalis model) where the male of one species actually punctures the body wall of the female of another species when mating with her. The consequences of this are fatal.

One paper was concerned with the attraction of tsetse males by their species-specific sex pheromone. This observation may lead to yet another means of enhancing the attraction of tsetse to insecticide-treated surfaces or to specific areas where sterility can be induced chemically.

A final paper in this session dealt with research and development undertaken at the Entomology Unit of the IAEA Laboratories at Seibersdorf in support of tsetse control programmes in Africa with SIT component. Emphasis was on semi-automation of tsetse rearing, procedures that offer potential for sex-differentiation in pre-adult stages and that might facilitate long-distance transport of pupae from a production centre across state borders.

Summary of Session on Vector Control (Part 2), Trapping, Use of Targets and Country Programmes

(Moderators/Rapporteurs - M. Hussain, B. Hursey and A. Van der Vloedt)

During this session, the scope and objectives of the Co-ordinated Research Programme on "Development of Controlled-release Formulations on Pesticides Using Nuclear Techniques" were outlined.

Results of laboratory tests were communicated in which UV absorber compounds, including substituted benzophenones, were evaluated for their efficacy in reducing photochemical decomposition of insecticides (e.g. alfa-endosulfan, fenthion, alfa-cypermethrin, cyfluthrin and deltamethrin) applied to cotton fabric. The beneficial effect of adding oils and other lipophilic materials to deltamethrin in reducing loss of insecticide from screens due to rain water was also documented.

Participants in the subject CRP communicated prelimary results of field testing and bioassays of controlled-release formulations of deltamethrin exposed to a variety of ecological conditions in Kenya, Tanzania and Ghana. The possible use of polymer mixture added to sprayable insecticides was also highlighted.

The majority of contributions during this session dealt with implementation aspects of ongoing national tsetse/trypanosomiasis control campaigns (Zimbabwe, Uganda, Botswana) and results of field testing of various trap/stationary target designs in combination with attractants (Ghana, Ethiopia, Kenya/Galana and Lambwe Valley, Uganda/Buvuma Island), the use of living targets treated with pour-on formulation and release of sterile males (Zanzibar). Some of the work reported is part of FAO TCP and/or IAEA TC projects. Discussions focussed on deployment and servicing of traps/targets under challenging conditions, and the need for closer supervision and increased community participation to avoid theft and damage of materials during operational tsetse control operations.

CONCLUDING REMARKS ON RESEARCH AREAS ON TSETSE CONTROL FOR FUTURE EMPHASIS

The development of practices for area-wide tsetse eradication or control with emphasis on suppression techniques and SIT component continues to be of major importance. Therefore, FAO and IAEA through their Joint FAO/IAEA Division and their specialized laboratory units (Insect and Pest Control Section and the Agrochemicals and Residues Section) should continue and even intensify to co-ordinate basic and applied research.

Although the SIT has been developed to the stage where it is a useful component of integrated tsetse control/eradication, it is very obvious that the technique must be made more robust, more reliable particularly in multispecies habitats, less costly and consequently more widely applicable.

It has been repeatedly pointed out (and also during the 1988 FAO Panel of Experts on Trypanosomiasis at Accra) that the cost of control operations with a SIT component is the most crucial factor limiting wide-scale use of the technique. Therefore, it would be desirable to develop a few fully equipped mass-rearing facilities for use on a regional and cost-shearing basis. From this point of view, it would be sensible to identify geographic zones in Africa where flies from a mass-rearing centre could be used across state borders in the most cost-effective way. The question of strengthening existing mass-rearing facilities in West Africa (e.g. Burkina Faso, Nigeria, Tanzania) or establishing one or two more production centres for example in East Africa needs to be considered logically. This would require careful planning and a realistic assessment of monetary costs and benefits.

It is also clear that the use of radioisotopes as tools for improvement of other tsetse control techniques should be fully explored.

Group discussions with the research contract and agreement holders during the week of 11-15 February 1991 highlighted a number of important research topics which were recommended for more in-depth study within the current or a new co-ordinated research programme. It has been suggested that molecular, biochemical cytogenetics and behavioural genetics of tsetse should be considered. These approaches should be applied to population genetics, particularly to monitor possible genetic changes in tsetse colonies and in tsetse populations that are subjected to control measures. Approaches to reproductive biology could emphasize hormonal control of reproduction and other phenomena that may be disrupted by genetic or chemical manipulations. Also, biochemical changes during reproductive cycles should be investigated because they may provide new approaches to suppressing tsetse. Relevant experimental approaches often would involve the use of radioisotopes. Many studies must be interdisciplinary.

Part I

DIAGNOSIS OF ANIMAL TRYPANOSOMIASIS

THE DEVELOPMENT AND APPLICATION OF ANTIGEN-DETECTION ENZYME IMMUNOASSAYS (ANTIGEN-ELISA) FOR DIAGNOSIS AND CONTROL OF AFRICAN TRYPANOSOMIASIS

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Abstract

Antigen-trapping enzyme immunoassays (Ag-ELISA) for the detection of circulating trypanosome antigens in the blood of infected animals were developed by ILRAD and the CTVM in response to a well-recognized need for improvement of the techniques for the diagnosis of African trypanosomiasis. With the conventionally used parasitological diagnostic methods, detection of the parasites is often curtailed by the fact that in the chronic phase of the disease, parasitaemia is intermittent and often not detectable.

The antigen-trapping ELISA systems which were developed have already shown encouraging results; the assay systems detect circulating trypanosome antigens, therefore patent infections. Applied to the diagnosis of bovine trypanosomiasis, a sensitivity of 96% was observed at ILRAD, and with good specificity. The assays are easy to perform, the results can be read visually if necessary and large numbers of sera can be analysed at a time.

A number of practical problems, such as transportability of biological reagents, the water quality required for the assay and the future assay quality assurance system are currently being addressed in the Network. Once these problems have been solved, the assays should be employed as a sensitive diagnostic tool in national tsetse and trypanosomiasis control programmes to determine the effectiveness of these programmes and to assess the efficacy and strategic use of trypanocidal drugs.

Introduction

The standard laboratory method for diagnosis of African trypanosomiasis is to demonstrate and identify trypanosomes in the blood of the infected host. There are several techniques for trypanosome detection which include direct microscopic examination of the blood either as wet film preparations or stained blood smears, the concentration techniques such as the microhaematocrit centrifugation technique (Woo, 1969) and animal inoculation. These techniques, however, are not sensitive enough as a high proportion of the infections go undetected (Barnett, 1947), particularly so in chronic infections which are often aparasitaemic (Masake & Nantulya, 1991). Moreover, the intermittent nature of the parasitaemia may preclude detection of the parasites even in the acute infections.

An alternative is to detect anti-trypanosome antibodies in the serum of the suspected case. For this, too, there are several techniques such as the indirect immunofluorescent antibody test (Williams et al., 1963), complement fixation (Shoening, 1924), direct and indirect agglutination (Magnus et al., 1978, Gill, 1966) and enzyme immunoassays (Voller et al., 1975). Antibody

detection, however, can only provide a presumptive diagnosis as it does not differentiate current from cured infections. Hence a positive antibody test should not necessarily form a basis for treatment of an animal as antibodies can stay in circulation for a long time after complete cure. This approach, however, is useful in epidemiological investigations.

Although undetectable in peripheral blood, trypanosomes can still be found sequestered in several other tissue organs of the infected host, such as the spleen, lymph nodes, bone marrow and liver. Successive trypanosome variable antigen types (VATs) in these populations are destroyed by the immune responses of the infected host as the individual mounts a desperate effort to eliminate the parasites. Several antigens are thus released in the tissue fluids, including some which are trypanosome species-specific. The detection of these antigens in a suspected case would thus provide direct evidence that the animal has a current infection. This is the rationale of the recently developed antigen-ELISA for diagnosis of Agrican trypanosomiasis.

Results and Conclusions

The assay is a sandwich ELISA in which trypanosome species-specific monoclonal antibodies are used to capture circulating trypanosome antigens in the serum of the infected host, either as a micro-ELISA plate assay (Nantulya, 1989; Nantulya & Lindqvist, 1989) or as a tube-ELISA (Nantulya et al., 1989b). The captured antigen is revealed by subsequent introduction of the same monoclonal antibody labelled with horseradish peroxidase. The labelled antibody will bind to the free antigenic sites (epitopes) of the trapped antigen, and the bound enzyme conjugate will be revealed by the introduction of substrate and chromogen. There are thus 3 tests, each of which is species-specific (T. congolense and T. vivax), or subgenous-specific (Trypanozoon).

In the initial evaluation of the assays using bovine field sera from a trypanosomiasis endemic area of Kenya, Nguruman, the 3 assays were able to detect trypanosome antigens in the sera of 121 (96.0%) out of 126 animals with parasitologically confirmed diagnosis. More importantly, the tests also detected antigens in 52.6% of animals in the same herd which had otherwise been missed by the buffy coat technique (Murray et al., 1977), emphasizing the superior sensitivity of these tests. Furthermore, the tests revealed that most infections (ca. 75%) in this series were actually mixed compared to 2.4% detected by the buffy coat technique. Finally, these assays were quite specific as bovine field sera from a trypanosomiasis-free area of Kenya, Kapiti Plains, all tested negative in the 3 assays. The most common parasitic diseases in the control herd were anaplasmosis (ca. 90%), theileriosis due to Theileria mutans (ca. 45%) and T. taurotragi (ca 10%) and babesiosis (ca. 40%).

An attempt was made to compare in more precise terms the sensitivity of antigen ELISA with that of the buffy coat technique (Murray et al., 1977). To do this, cattle and goats were infected experimentally and the two assays compared with regard to their ability to detect the trypanosomes or the antigens in repeated blood samples obtained at monthly intervals from the infected animals. The results obtained indicate that for T. congolense which has already been analyzed, the sensitivity of antigen-ELISA was at least 4 times higher than the buffy coat technique (Masake & Nantulya, 1991). The 3 assays are currently being evaluated in several African countries, in a collaborative project involving FAO, IAEA and ILRAD for diagnosis of bovine trypanosomiasis. This project is funded by the Government of the Netherlands.

The assay for T. brucei does detect an antigen expressed by the human-infective trypanosomes T. b. gambiense and T. b. rhodesiense (Nantulya et al., 1987), T. evansi (Nantulya et al., 1989a, b) and T. equiperdum (T. Baltz, personal communication). Thus, this test is currently under evaluation in Côte d'Ivoire and Zaire for diagnosis of T. b. gambiense sleeping sickness, and in Zambia, Tanzania and Uganda for diagnosis of T. b. rhodesiense, under the sponsorship of the UNDP/World Bank/WHO Tropical Disease Research and Training Programme. The results so far obtained indicate the test to have a high degree of sensitivity (90-95%) and specificity (99.9%) (Nantulya et al. and Komba et al., in preparation). The sensitivity observed in the diagnosis of T. evansi infections in camels (Nantulya et al., 1989b) and T. equiperdum in rabbits (T. Baltz, personal communication) was close to 90%.

In summary, the following observations need emphasis: (1) the 3 tests detect current infections and are hence synonymous with parasitological diagnosis; (2) the tests are specific, sensitive and simple to carry out; (3) because host species' immunoglobulin - specific reagents are not required, a single test can be used for diagnosis of the disease in several animal species and (4) the results from the field indicate that these assays have a high potential for improving diagnosis of African trypanosomiasis. Nevertheless, there are practical problems to be overcome, namely, the sensitivity of the enzymes to water quality, cost and standardization. All these problems are currently being addressed in the design of improved techniques for field use.

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APPLICATION OF ANTIGEN-ELISA FOR THE DIAGNOSIS OF SURRA IN SELECTED CAMEL HERDS IN KENYA

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Abstract

Kenya has about one million dromedary camels. These are kept in the arid and semi-arid areas of the eastern and north-eastern provinces of the country. The dromedary camel plays an important role in the livelihood of the nomadic pastoral tribes of Kenya, where they are mainly used for milk, meat and as draft animals.

Of the diseases affecting camels in Kenya, trypanosomiasis due to Trypanosoma evansi is the most important. The disease causes mortality of up to 70%, abortions and weight losses in affected herds. Measures aimed at controlling the disease have always been hampered by the lack of sensitive diagnostic tests. In recent years, the application of enzyme immunoassays has greatly improved the diagnosis of trypanosomiasis. In an antigen-ELISA test which employs a monoclonal antibody, we were able to detect more than 90% of patent infection in affected camels. The test is sensitive, specific and easy to perform. The test is very promising in the diagnosis of patent and cryptic trypanosome infection. The significance of the results is discussed.

Introduction

Of the protozoal diseases affecting camels, trypanosomiasis due to Trypanosoma brucei evansi is the most important (Leese, 1927). T. b. evansi infection in camels is characterized by abortions, loss in production due to reduced milk output and bodyweight loss and mortality in calves and susceptible adults. In Kenya, the impact of the disease has been studied only in selected herds (Wilson et al., 1981; Olaho-Mukani et al., 1981). However, the assessment of the full impact due to the disease in camels has been difficult to arrive at accurately in the past, due to lack of suitable diagnostic methods which can determine the magnitude of its prevalence, incidence and morbidity. Recently, Nantulya et al. (1989) described two simple antigen-detection enzyme immunoassays for the diagnosis of T. b. evansi in camels. The techniques employ the use of a monoclonal antibody in a sandwich ELISA. We also report the employment of a monoclonal sandwich ELISA in the diagnosis of T. b. evansi infection in selected study herds in Kenya.

Materials and Methods

Camel Herds

Camel herds sampled included: (i) Athi-River, (ii) Kisima in Eastern Province and (iii) Kajiado in Rift-Valley Province of Kenya.

Parasitological Techniques

Parasitological techniques used in the study included the haematocrit centrifugation technique (HCT) and the rodent sub-inoculation test.

Enzyme Immunoassay

Monoclonal antibody TEA 1/23.4.6 was used to couple micro-ELISA plates as described by Olaho-Mukani (1989). Briefly, 10 ul of the monoclonal antibody diluted to 25 ug/well in carbonate/bicarbonate buffer, pH 9.6, were added to each well of the micro-ELISA plate. The plates were then emptied, washed three times with 0.01 m phosphate-buffered saline, pH 7.4 (5 minutes/wash) and dried on absorbent paper. To each micro-well, 100 ul of undiluted serum or plasma was applied and plates incubated at 37°C for one hour. The washing and drying process was repeated as above, before 100 ul per well of TEA 1/23.4.6-peroxidase conjugate diluted 1:1000 was added and the incubation process repeated at 37°C for one hour. Thereafter, the plates were washed and dried as before and then orthophenylene diamine (OPD, Sigma, USA) was added at 100 ul per well and plates incubated at room temperature in the dark for 30 mintues. The enzyme-substrate reaction was stopped by adding 50 ul/well of 1.0 m sulphuric acid and optical density readings taken in a Dynatech micro-ELISA autoreader (MR 580) at a wavelength of 492 nm. All samples were tested in duplicates. Optical density values above 0.09 were taken as positive as described by Olaho-Mukani (1989).

Treatment of Camels

The Athi-River male herd camels were treated with quinapyramine prosalt according to the instructions of the manufacturers. The camels were again sampled on days 14, 28 and 48.

Results

Parasitological and Serological Results

Table 1 shows the results of parasitological and serological examination. Antigen-ELISA detected between 94% and 100% of patent infection in the affected herds. The analysis of variance and multiple range testing, based on Athi-River male herd, showed a high statistical significance ($p<0.0001$) between infected and non-infected Ag-ELISA OD values for both serum samples and plasma samples (see Table 2). The highest incidence of patent infection was demonstrated in the Athi-River male herd, followed by the Kajiado camels. These results were well reflected in the Ag-ELISA values for these herds. Both the Athi-River female herd and the Kisima herd had very low infection rates.

Table 1. Results of parasitological and Antigen-ELISA testing

Camel Herd	% Parasite Positive	% Ag-ELISA Positive	% Parasite Positive detected by Ag-ELISA
Athi-River Female Herd (n=83)	0	4.8	N/A
Athi-River Male Herd (n=55)	85	85	94
Kajiado camels (n=94)	19	60	100
Kisima camels (n=102	1	8	100

Table 2. Anaylsis of variance and multiple range testing of mean (x±SE) Ag-ELISA values of infected and non-infected camels at Athi-River (n=55)

		df	MS	F	(P)
A. Serum Ag-ELISA:	Between groups:	1	1.036	45.03	(<0.0001)
	Within groups:	218	.023		
	Group Means				
	Infected		Non-infected		
	.268±.026		.102±.011		
B. Plasma Ag-ELISA	Between groups:	1	1.385	39.25	(<0.0001)
	Within groups:	218	.035		
	Group Means				
	Infected		Non-infected		
	.321±.033		.129±.0133		

Circulating Antigen Profile

Figure 1 represents the profile of circulating antigens in a typical herd with low infection rate, characterised by low Ag-ELISA values. Figure 2 represents a herd with high infection rate (Kajiado camels) characterized by a well spread out range of Ag-ELISA OD values.

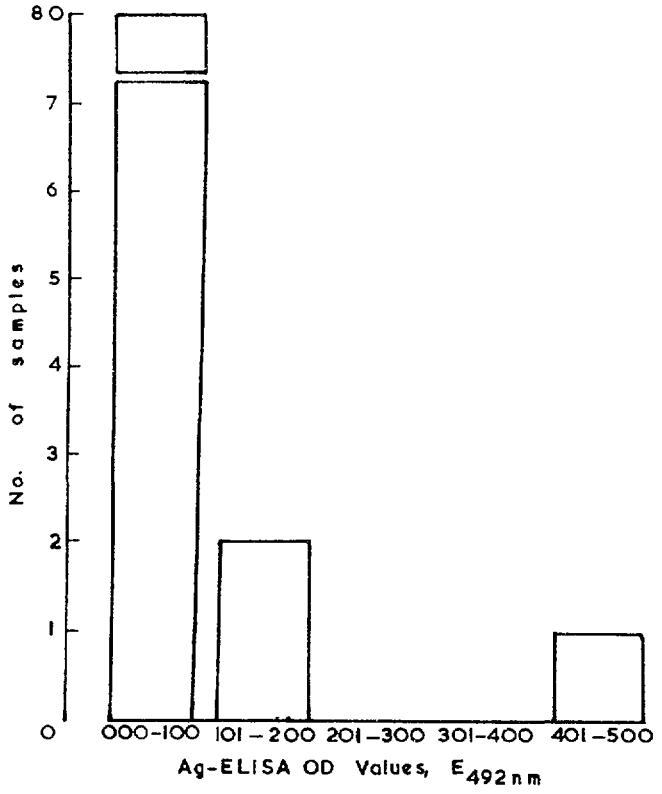

Figure 1. Circulating antigen profile in the Athi-River female camel herd

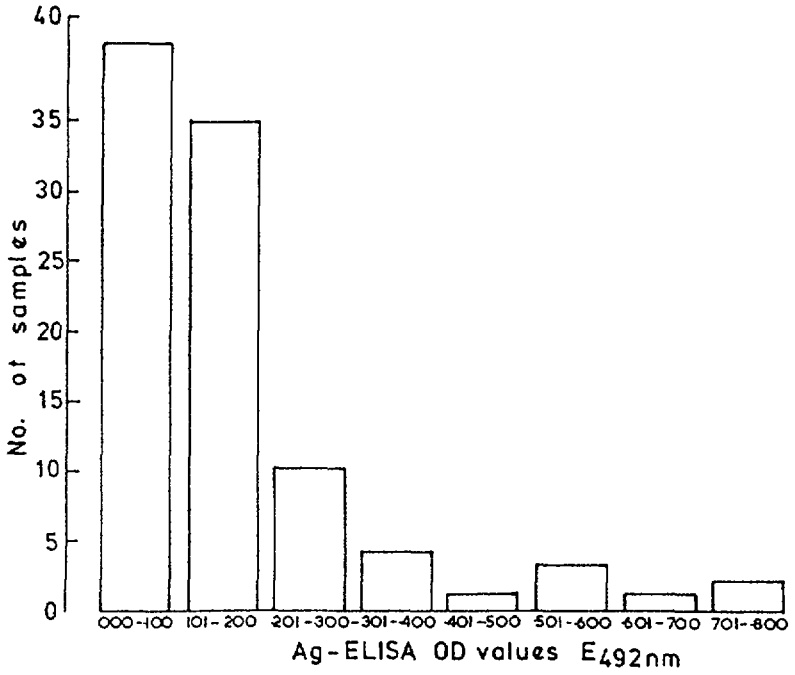

Figure 2. Circulating antigen profile in the Kajiado camels

Circulating Antigen Profile Following Treatment

The circulating antigen profile following treatment is shown in Figure 3. The majority of the camels showed a drop in antigen levels below the lower limit for positive values by day 28. However, in a few camels, antigen levels dropped and then rose again, while in others, the levels remained high as shown in Figure 4.

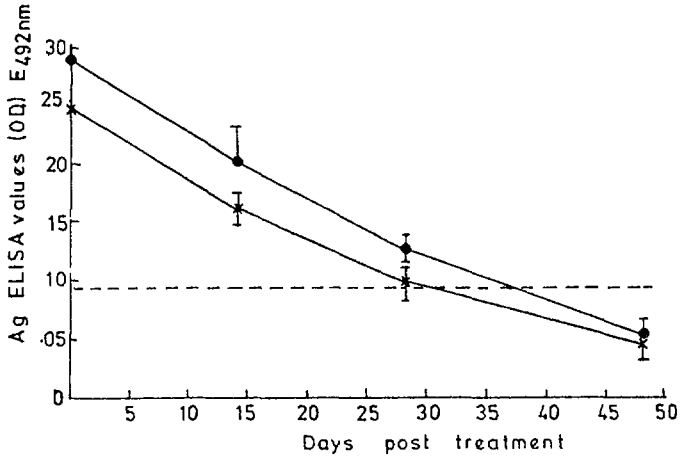

Figure 3. Plasma (●——●) and Serum (x——x) Ag-ELISA values (OD, E_{492nm}) in the Athi-River male camels (----------) = Lower limit for positive Ag-ELISA

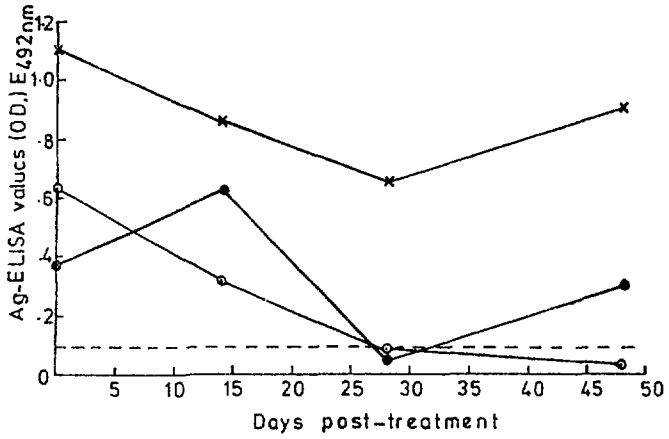

Figure 4. Circulating antigen levels (OD,E_{492nm}) in Athi-River Male camels after treatment:

x——————x = no drop below the negative limit
0——————0 = dropped below the negative limit
●——————● = dropped below the negative limit and became elevated again

Discussion

In the past, the diagnosis of Surra was based mainly on clinical observations and the detection of parasites by parasitological techniques. Often, these tests were supported by the indirect methods of antibody detection in sera. However, the shortcomings of these approaches have been obvious for many decades, because the disease has no pathognomonic signs, the parasites may be too scanty in peripheral circulation to be detected and antibody detection does not relate to patent infection with meaningful precision (Gatt-Rutter, 1967; Killick-Kendrick, 1968; Luckins, 1988).

In the present work described, the Ag-ELISA detected 94-100% patent infection and showed that in the non-infected herds the values remained negative. The profile of circulating antigen levels shows a sharp drop after treatment with a prophylactic drug to below the negative levels by day 28 post treatment. This compares well with the findings of Rae and Luckins (1984), Liu et al. (1988) and Olaho-Mukani (1989).

The disappearance of circulating trypanosomal antigens may be an indication of successful chemotherapeutic intervention, while persisting circulating antigens may be an indication of persisting parasitaemia in cryptic foci. Thus, the Ag-ELISA technique may be an ideal technique for evaluating the success of therapeutic intervention in the control of trypanosome infection.

Thus, these results clearly show that the Ag-ELISA technique can be used to supplement parasitological techniques. If this test is carried out as described by Nantulya et al. (1989), its field application is quite possible.

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VALIDATION OF THE ELISA TECHNIQUE FOR DIAGNOSIS OF TRYPANOSOMIASIS IN CATTLE IN UGANDA

(Summary)

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ELISA, developed in ILRAD for diagnosis of T. congolense, T. brucei and T. vivax in cattle, has not been validated in Uganda. This study was undertaken to validate the technique.

Negative reference sera were collected from 44 cattle in Kapchorwa, a tsetse-free area. The cattle were free of the three trypanosome species T. congolense, T. brucei and T. vivax by the haematocrit buffy coat technique (BCT). But by ELISA, three were positive for T. vivax, one for both T. congolense and T. vivax and one for T. congolense.

Sera were collected from the same 44 cattle 10 weeks later. The cattle were again free of T. congolense, T. brucei and T. vivax, both by BCT and by mouse inoculations. Two cattle out of 450 screened at a centre 5 km away had T. vivax by BCT. The ELISA results for the second set of sera were quite similar to the results obtained from the first set of sera.

The calculated optical density (OD) cut off point was 50 for both T. brucei and T. vivax, but it was 60 for T. congolense. Sera from 5 cattle which had T. theileri and two which had microfilaria were all negative for antigenaemia by ELISA.

Positive reference sera were collected form 40 cattle in a high tsetse challenge area. Using the haematocrit buffy coat technique, 5 had T. vivax, two had T. brucei and one had T. congolense. Checked by ELISA for antigenaemia, only 4 cattle were free of all the three trypanosome species, T. congolense, T. vivax and T. brucei. All the 40 cattle were treated with Diminazene aceturate at the rate of 7 mg/kg body weight. Two weeks later, the ELISA test showed that 10 cattle were free of any antigenaemia. Those still positive for antigenaemia had lower OD readings.

The ELISA technique is valid. It is much more sensitive compared to parasitological tests. It is specific since none of the 7 cattle with either T. theileri or microfilaria gave positive results by ELISA. The technique would be very useful for epizootiological studies.

CURRENT STATUS OF IN VITRO ASSAYS FOR IDENTIFYING DRUG-RESISTANT TRYPANOSOMES

(Summary)

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Recent progress in development of in vitro assays to assess drug susceptibility in African trapanosomes of veterinary importance will be reviewed. In most cases, different drug susceptibilities of trypanosomes in cattle or mice correlate with different susceptibilities to drugs in in vitro cultures when bloodstream or metacyclic forms are used. The criteria in these assays to distinguish between susceptible and resistant trypanosomes are infectivity, metabolism (^{3}H-hypoxanthine incorporation), growth (24-hour growth inhibition) or death/survival in long term cultures. Some of the assays require the adaptation of isolated trypanosomes to continuously growing in vitro cultures. Other factors important for the application of the assays include the time period required from isloation to the end of the test and the laboratory equipment necessary to perform the assays.

The use of insect form trypanosomes has several advantages over the use of bloodstream form parasite. Most T. b. brucei and T. congolense trypomastigotes are easily transformed into procyclics forms in vitro and can be maintained arsenically in log phase growth. However, in many cases, drug-induced inhibition of growth or ^{3}H-hypoxanthin incorporation does not correlate with different drug susceptibilities in vivo.

PHARMACOKINETICS OF DIMINAZENE ACETURATE (BERENIL^{R}), HOMIDIUM BROMIDE (ETHIDIUM^{R}) AND ISOMETAMIDIUM CHLORIDE (SAMORIN^{R}) AFTER INTRAVENOUS APPLICATION IN BORAN STEERS

(Summary)

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The trypanocides Berenil^{R}, Ethidium^{R} and Samorin^{R} are routinely applied intramuscularly (i.m.), however, due to the increase in drug resistance of trypanosomes, some investigators and farmers have adopted the intravenous (i.v.) route and have claimed its superiority. This study establishes the pharmacokinetics of these trypanocides after i.v. application and compares it with the pharmacokinetics after i.m. application.

^{14}C labelled trypanocides were administered intravenously to Boran steers and the radioactivity levels were determined in plasma, tissue fluid, urine, faeces and tissues.

The peak plasma levels of Berenil^{R} and Ethidium^{R}, after i.v. application, were approximately three to seven times higher than after i.m. application. With Samorin^{R} they were between 18 and 36 times higher using the intravenous route. The decline of plasma levels after i.v. treatment showed two phases with all three drugs, with the level of the second phase being similar after i.v. and i.m. treatment.

The tissue fluid levels were lower than the plasma levels after i.v. treatment with Berenil and Ethidium, however, they were higher than the plasma levels following the i.v. treatment with Samorin.

The excretion rates were initially higher after i.v. treatment. However, the accumulated excretion was similar already ten days post treatment with Berenil and Ethidium but ten days after i.v. treatment with Samorin, two-fold higher and still approximately 50% higher sixty days post treatment.

The residue level in tissue was higher after i.v. treatment.

These results show that Samorin is the only drug showing higher tissue fluid than plasma levels and that after intravenous treatment higher initial peaks are achieved, which especially with Samorin increase the curative effect in areas with resistant strains. However, the high skill required to use intravenous administration for trypanosomiasis control will limit its usage.

INVESTIGATIONS OF INTRAMUSCULARLY INDUCED DIMINAZENE ACETURATE (BERENIL^{R}) PLASMA LEVELS IN CATTLE UNDER HIGH TSETSE AND TRYPANOSOME CHALLENGE IN THE FIELD

(Summary)

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The prophylactic effect of trypanocides is significantly reduced under high fly and trypanosome challenge. It was assumed that this is due to a reduction of drug plasma levels by trypanosomes at the time of treatment and challenge.

Cattle herded at the Kenya coast under high fly and trypanosome challenge and cattle herded at KETRI without fly and trypanosome challenge were treated repeatedly with Berenil^{R} and the plasma levels established using the HPLC technique.

Comparing both groups of cattle, it was found that neither the height of the initial peak nor the half lives of the plasma levels were significantly different.

A COMPARISON OF INTRAMUSCULARLY INDUCED PLASMA LEVELS OF DIMINAZENE ACETURATE (BERENIL^{R}), HOMIDIUM BROMIDE (ETHIDIUM^{R}) AND ISOMETAMIDIUM CHLORIDE (SAMORIN^{R}) IN INFECTED AND UNINFECTED CATTLE

(Summary)

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The prophylactic effect of trypanocides is significantly reduced under high fly and trypanosome challenge. It was assumed that this might be due to a reduction of drug plasma levels by trypanosome parasitaemia.

The ^{14}C labelled trypanocidal drugs Berenil^{R}, Ethidium^{R} and Samorin^{R} were administered intramuscularly in uninfected and in cattle infected with Trypanosoma congolense. The radioactivity levels were determined in plasma.

The results indicated a slight but non-significant reduction in initial plasma level in infected cattle. Therefore, it can be concluded that the amount of drug trypanosomes are taking up is not influencing the plasma level and therefore is not lowering the curative or prophylactic effect.

SORBENT EXTRACTION AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) OF HOMIDIUM BROMIDE AND ISOMETAMIDIUM CHLORIDE IN BOVINE PLASMA

(Summary)

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Homidium bromide and isometamidium chloride are used extensively in the treatment of bovine trypanosomiasis in Africa, but no specific and sensitive method to detect the drug in plasma has been published yet.

Comparing the methods of other investigators it was found that they could not overcome the protein binding of these drugs which reduced the available and determined drug to 10% of the real concentrations.

These techniques overcome the protein binding by enzyme digestion and an alteration of the pH before adding the sample directly on clean-up columns. Drug recover rates above 80% were obtained.

For the detection, the HPLC was employed using a C18 reversed phase analytical column and UV detection, determining both drugs as intact molecules.

Part II

CHEMOTHERAPY OF ANIMAL TRYPANOSOMIASIS

SOLID PHASE EXTRACTION AND REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF HOMIDIUM IN ANIMAL TISSUES

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Abstract

Homidium bromide, an antitrypanosomal drug, has been in use for about 40 years both for chemotherapy and limited chemoprophylaxis but no specific and accurate method for its detection at sub-microgram levels in tissues is available.

The HPLC method described uses C18 reversed phase analytical column with UV detection after basic sample extraction and clean-up on a 3 ml cyano Bond Elut disposable extraction column.

Drug recoveries of up to 80% were obtained in the extracts. All tissues analysed were from animals treated with ^{14}C labelled homidium at a dose of 1 mg/kg body weight and slaughtered at 14, 21 and 28 days after treatment.

Recoveries were determined by comparing radiometric and HPLC results. The advantage of this procedure was that large amounts of samples could be processed especially from tissues with low drug levels. The detection limit was 50 ng per gram wet tissue. This method is simple, fast, accurate and sufficiently sensitive to be used for monitoring drug levels in meat destined for human consumption.

Introduction

Apart from its use as a trypanocidal drug, homidium bromide has been widely used as a biochemical probe (Newton, 1976) due to its molecular interaction with DNA. Its pharmacokinetics have only been studied by use of ^{14}C labelled drug (Newton and Gilbert, 1981).

Generally, trypanocidal drugs are rather toxic to the host and doses tolerated are usually low. Because of the low dosages, the amounts distributed in the blood and tissues have been difficult to determine by chemical means. Radiometric methods, though sensitive, lack specificity since the total active species is determined which may not necessarily be an intact drug. The spectrophotometric method used by Kandaswany and Henderson (1963) is not sensitive enough to determine the sub-microgram levels of the drug in tissues. A fairly accurate, sensitive and specific method has therefore been developed for the determination of homidium bromide in animal tissues.

Materials and Methods

All chemicals and reagents used were of analytical grade. Water was distilled and then filtered.

Tissue samples analysed included liver, kidney, heart, muscle and spleen obtained from animals treated with ^{14}C labelled drug and slaughtered at 14, 21 and 28 days after treatment. Samples were homogenized using an Ultra Turrax tissue homogenizer.

To 1 g homogenate were added 5 ml of concentrated hydrochloric acid, digested on a waterbath for 10 minutes, cooled immediately and neutralized with 50% sodium hydroxide. The solution was then made definitely alkaline by adding excess sodium hydroxide (about 2 ml) to pH 10-11. The digest was then extracted twice with ethyl acetate (2 x 10 ml). Ten ml of the pooled extracts were loaded onto a cyano Bond Elut 3 ml disposable column preconditioned with 3 ml methanol and 5 ml ethyl acetate.

After passing all sample through, it was washed with 3 ml ethyl acetate, evacuated, then washed with 2 ml 60% acetonitrile and 3 ml water. The drug was then eluted with 3 ml 50% methanol containing 10 mg per ml ammonium chloride. After rejecting the 1st 990 ul, 1 ml was collected for analysis both by radiometric detection and HPLC. Injection volume for HPLC was 200 ul.

For enzyme digested samples, 20% homogenates were prepared in 0.1 M borax buffer pH 10.5. To 5 ml homogenate were added 3 mg enzyme Subtilisin Carlsberg, incubated at 56°C for 2 hours and then extracted with ethyl acetate after addition of 2.0 g sodium chloride and 1 ml 50% sodium hydroxide.

Chromatography

A Waters HPLC system was used consisting of a Model M-45 constant flow delivery pump, a 6 UK injector with a 2 ml sample loop. The column effluent was monitored with a variable wavelength Lambda Max Model 480 UV detector at an absorption wavelength of 292 nm.

The analytical column was a 25 cm long 10 um C18 Polygosil column fitted with a guard column. The mobile phase consisted of methanol and 0.6% v/v acetic acid in the ratio of 45:55, to which had been added 1 mg ammonium chloride per ml solution, sonicated and pumped at the rate of 1.5 ml min^{-1}. Peak areas were determined by an on-line Waters QA-1™ data system. Standards were prepared in water.

Results

Using the above procedure, homidium was well separated by HPLC with a retention time of 5.2 minutes.

Linearity was established with standards up to 500 ng (Figure 1) with a limit of detection of 2 ng.

Table 1 gives the expected concentrations of homidium in nanograms per gram wet tissue obtained at different intervals post-treatment calculated from total radioactivity measurements and showing the distribution of the drug in the organs studied.

Table 2 shows the distribution of the drug in muscle of one animal.

Table 3 compares drug recoveries obtained under different sample treatments.

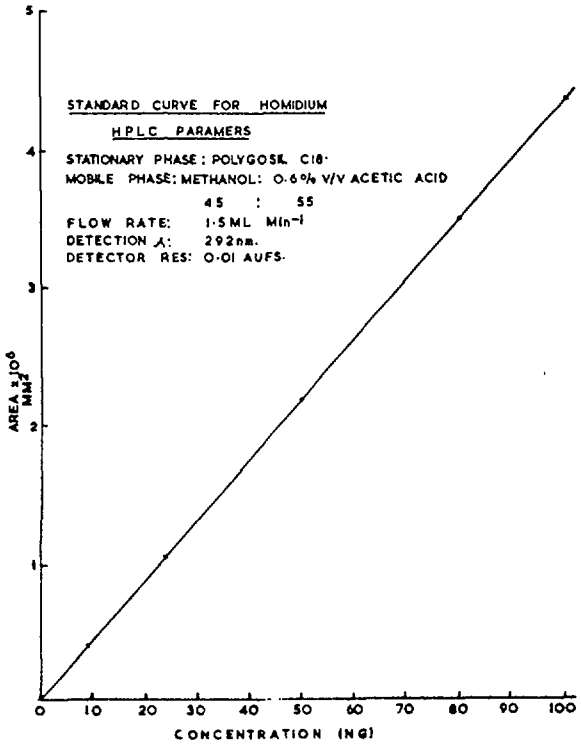

Figure 1.

Table 1. Expected homidium bromide concentration in nanograms per gram wet tissue from total radioactivity measurements at various periods after treatment.

Tissue Type	Concentration (ng/g)		
	14 Days 1 mg/kg i.m.	21 Days 1 mg/kg i.v.	28 Days 1 mg/kg i.m.
Heart	34.48± 1.19	39.67± 1.00	11.94± 0.86
Spleen	54.14± 1.47	102.99± 0.26	30.64± 0.79
Muscle	118.19± 2.10	302.90± 3.91	76.15± 0.65
Kidney	400.17±16.94	785.71±11.10	366.09±11.15
Liver	755.39±29.57	2614.67±43.85	657.79±56.61

Table 2. Muscle distribution of homidium bromide in boran steer 14 days post-treatment.

	Drug Concentration (ng/g)
Left Fore-leg	253.24±0.67% s.d.
Right Fore-leg	331.46±2.92% s.d.
Right Hind-leg	248.08±1.85% s.d.
Left Hind-leg	405.66±2.34% s.d.

Table 3. Drug recoveries obtained from muscle under different sample treatments.

Treatments	Mean % Recovery (±s.d.)
pH 7.5 + enzyme	72.84±4.00
pH 7.5 no enzyme	40.33±6.00
pH 10.5 + enzyme	82.74±6.56
pH 10.5 no enzyme	36.98±3.93
Acid Digestion	80.19±2.43

Table 4 gives % recoveries of the drug in the ethyl acetate extracts calculated from radioactivity measurements.

Table 5 gives drug recoveries from tissue spiked with different drug concentrations.

Table 4. % drug recoveries in the ethyl acetate extract at different periods post-treatment.

Tissue Type	14 Days	21 Days
Heart	80.75± 7.54	80.51±10.95
Spleen	66.41± 9.79	38.00± 7.25
Muscle	80.19±11.19	79.37±12.82
Kidney	67.07± 3.80	48.85± 6.40
Liver	37.32± 5.54	37.78± 3.60

Table 5. Spiked samples: comparison of drug recoveries between radiometric and HPLC methods.

Amount of Drug Added Per Gram Tissue	% Recovery Radiometric	% Recovery HPLC	Radiometric HPLC
50	54.14±6.42	51.82±1.16	1.04
100	50.23±0.16	46.52±7.19	1.08
250	63.14±4.93	59.47±8.90	1.06
500	70.19±2.40	68.07±8.80	1.03

Figures 2 to 4 show chromatograms obtained from HPLC for the various tissues.

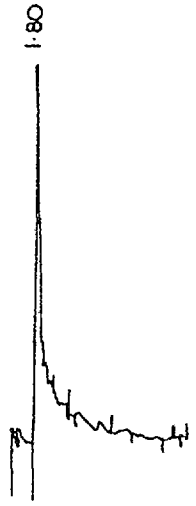

Figure 2. Blanc tissue.

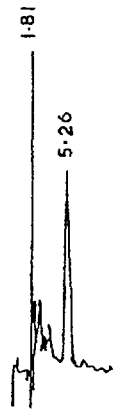

Figure 3. Muscle tissue three weeks after intravenous administration at homidium bromide at a dosage of 1 mg/kg body weight.

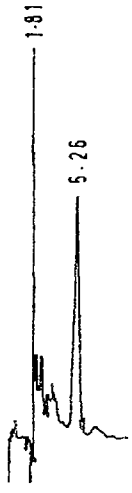

Figure 4. Kidney tissue three weeks after intravenous administration of homidium bromide at a dosage of 1 mg/kg body weight.

Table 6 compares radiometric and HPLC results for the various tissues.

Table 6. Comparison of % drug recoveries: radiometric vs. HPLC detection methods.

Tissue Type	% Recovery Radiometric	% Recovery HPLC	Radiometric HPLC
Heart	58.14±3.78	ND	-
Spleen	74.99±4.03	36.90±3.03	2.03
Muscle	79.67±7.00	57.65±5.20	1.38
Kidney	62.38±2.22	39.59±4.72	1.58
Liver	36.16±2.57	14.81±2.76	2.44

Discussion

With this sensitive method it was possible to detect the drug in very low quantities. Limit of detection was 50 ng per gram wet tissue.

The results above give a picture of the type of drug levels expected and how these can vary significantly even in the same animal. All levels are sub-microgram apart from liver of the animal treated intravenously. These levels have actually been known to protect the animal depending on the fly challenge (Kratzer et al., unpublished information), the prophylactic period ranging between 7 and 21 days.

It was therefore necessary to establish a procedure that would give high drug recoveries without decomposing the drug. Though high recoveries were established with enzyme digested samples, the samples eluted after the clean-up procedure remained too dirty to be chromatographed. Eluates from acid digested samples, however, were cleaner through the same clean-up procedures. Spiked tissues, when treated with acid, showed that the drug does not decompose during treatment and subsequent extractions.

The concentrations obtained by radioactivity measurement were comparable to those obtained by HPLC for intact homidium.

Though some tissues gave as low recoveries as 37%, the method was sensitive enough to be able to detect the drug.

When radiometric and HPLC results obtained from experimental animals were compared, several differences were noted. Drug levels in heart were too low to be detected by this method whose detection limit was 50 ng/g wet tissue. Spleen which had high radioactivity had only traces of homidium suggesting that the activity was possibly due to metabolites. Interestingly, most of the activity measured in muscle tissue was due to unchanged homidium suggesting a rather low level of metabolites. For liver and kidney, it appeared from the results that half of the activity measured was due to homidium and the rest possibly being metabolites.

Conclusion

The use of enzyme makes analysis expensive, especially if used for routine analysis. In the procedure described, the use of enzyme has been avoided.

Radiometric procedure determines total active species in solution which may not be the molecule being sought.

Comparison between HPLC and radiometric detection revealed that only half of what was detected by radiometric method was homidium, the rest possibly being metabolites. Newton and Gilbert (1981) found that when urine and bile were chromatographed, up to 46% radioactivity separated from an ethidium marker. Two excretion products were detected but were not identified.

Kandaswany and Henderson (1963) detected no metabolites in mice tissues 24 hours post-treatment.

It is therefore not known what part these metabolites play in the treatment and prophylaxis of animal trypanosomiasis until they have been identified and isolated for trials.

The analytical method described is simple, fast, accurate and sufficiently sensitive to be used (a) for routine analysis, (b) in monitoring homidium drug residues in meat destined for human consumption, (c) in the study of metabolites and their role in the treatment and prophylaxis of animal trypanosomiasis and (d) in the study of drug resistance attributed to homidium.

Acknowledgements

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METABOLITE STUDIES OF ISOMETAMIDIUM IN CATTLE

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Abstract

Isometamidium, a phenathridinium compound, has been used extensively both for prophylaxis and therapy against trypanosomiasis in cattle. The lack of detection of this drug in serum and urine within 24 hours following either i.v. or i.m. administration of the non-labelled drug as opposed to the labelled drug, would suggest either insensitivity of the methods used or rapid metabolism of the drug to compounds which are active against trypanosomes at these concentrations.

A preliminary study was carried out using liver, kidney, bile, blood and urine samples from two groups of steers, one of which had been treated with ^{14}C-isometamidium labelled on the homidium molecule and the other with ^{14}C-isometamidium labelled on the benzamidine molecule and slaughtered 21 and 60 days after treatment.

Isometamidium was detected in the liver up to 21 days post treatment but was not detected at 47 and 60 days post treatment. Metabolites which corresponded to homidium and benzamidine derivatives were detected in liver, kidney, bile and urine samples. No isometamidium was detected in urine collected 24 hrs after treatment. From this study, it seems that the prophylactic activity of isometamidium is due to homidium and/or its derivatives.

Introduction

Isometamidium (ISMM), a major constituent of Samorin^R, is used extensively both for treatment and prophylaxis of trypanosomiasis in cattle. It is effective against both Trypanosoma congolense and Trypanosoma vivax. Isometamidium is unstable in acidic medium and at elevated temperatures. It degrades to homidium and various derivatives of amidino phenyl diazonium salt depending on the reaction conditions (i.e. pH, temperature and light). In fact, Perschke and Vollner (1985) used this property in their method for the determination of Samorin^R in plasma. Hydrochloric acid was used to break down ISMM and was determined as homidium by HPLC. Although ISMM has been used in the field for about 30 years, knowledge of its therapeutic and prophylactic activities in relation to its pharmacokinetic properties is not properly known.

In cattle, ISMM was found to be cleared from serum within 4 - 8 hrs to concentrations below 10ng/ml (Kinabo & Bogan 1988). However, in other animal species, goats (Braid & Eghianruwa 1980) and camels (Ali & Hassan 1984), sub-micro levels of the drug were found in plasma 24 hrs after treatment. This discrepancy could be attributed to the sensitivity of the different analytical methods used or differences in elimination mechanisms between animal species. Studies using radiolabelled ISMM have shown significant amounts of activity in plasma after six weeks and in tissues after six months (Kratzer, unpublished data). Since this activity cannot really be assigned to the intact drug alone, the lack of detection of ISMM using the "cold" methods would indicate either rapid elimination of the drug from the body or rapid metabolism of the drug to products which have trypanocidal activity at those concentrations.

However, following parenteral administration of ISMM in cattle (Kinabo & Bogan 1988) and in rats (Phillips et al. 1967), no other compounds were detected in plasma or serum as putative metabolites, except after oral administration of the drug in rats (Phillips et al. 1967) when it was found to break down to homidium due to the acidic environment in the stomach.

Investigation under controlled conditions have revealed that homidium, an analogue of ISMM, is metabolized in rats primarily by N-acetylation in the liver and is excreted directly into the bile (Macgregor & Clarkson 1971). Similarly, in vitro studies have shown that homidium and a number of other phenanthridinium compounds undergo bio-transformation by rat liver metabolizing enzyme system (Macgregor & Clarkson 1977; Lecointe et al. 1981). Whether ISMM also undergoes N-acetylation in the liver is not clear, but it was found to be excreted in faeces via the bile (Phillips et al. 1967) probably through the same mechanism demonstrated for homidium and other quarternary ammonium salts.

It was the purpose of this study to determine the presence of metabolites in cattle after Samorin^{R} treatment and try to establish their role and contribution in the efficacy of ISMM as a prophylactic drug. Preliminary results of this study are reported in this paper.

Materials and Methods

Two groups of steers which had been treated with 1 mg/g of ^{14}C-Samorin^{R} were used in this study. In one group, Samorin^{R}(I) labelled at the homidium molecule was used while in the other group, Samorin^{R}(II) labelled at the benzamidine moeity was used for treatment. Three animals were slaughtered after sixty days while two were slaughtered after 21 and 47 days respectively. Kidney, liver and urine samples were analysed by HPLC using methods developed in this laboratory. Radioactivity in the samples was measured using a Beckman Liquid Scintillation Counter. Thin layer chromatography was also performed on all the samples. 20 ul of the organic extract of the samples were spotted on pre-coated flourescence silica gel plates. The solvent system used was a mixture of chloroform, acetonitrile, acetic acid, formic acid and water in the ratio 35:40:10:10:2. The plates were viewed under UV light at 265 nm. Liver, kidney and urine samples from untreated animals were used as controls.

Results

In both kidney and liver (Table 1), isometamidium and homidium could be detected 21 days after treatment, but could not be detected 47 days and 60 days after treatment. At 21 days, two unidentified peaks (rt. 4.45 and rt. 8.11) which had activity were present in the liver while only one unidentified peak (rt. 4.45) was detected in the liver. At 47 days and 60 days 3 more unidentified peaks (rt. 2.06, rt. 2.46 and rt. 3.20) were observed both in the liver and kidney (Table 2). Two peaks out of the five unidentified peaks from the two groups of steers had activity.

Discussion and Conclusion

The presence of ISMM in both liver and kidney at 21 days agrees with what was observed by Kinabo & Bogan 1988, who found the drug in both kidney and liver up to 42 days post-treatment. It may seem that between 42 days and 47 days the ISMM concentration in the liver disappears rapidly to non-detectable levels. It is interesting to note that the amount of ISMM in liver were approximately half the concentration in the kidney in contrast to the observations by Kinabo & Bogan 1988, while that of homidium was the same in

Table 1. % activity of the peaks detected on HPLC from the livers and kidneys of the different animals analysed

		Liver		Kidney	
		Peaks rt. (min)	% act.	Peaks rt. (min)	% act.
SAMORIN I					
	Animal 1	4.45	9.6	5.03	19.1
	21d P/T	5.03	24.9	8.11	26.3
		8.11	34.3	8.75	25.6
		8.75	12.1		
	Animal 2	2.05	0.0	2.46	5.1
	60d P/T	2.46	4.3	3.20	15.2
		4.45	22.4	4.45	6.0
		8.11	38.5		
SAMORIN II					
	Animal 1	3.20	22.1	2.07	16.5
	47d P/T			3.20	2.1
				4.45	0.0
	Animal 2	2.46	8.4	2.06	20.2
	60d P/T	3.20	15.9	2.46	9.3
		4.45	0.0	3.20	16.1
				8.11	0.0
	Animal 3	4.45	0.0	2.06	19.1
	60d P/T	8.11	0.0	2.46	21.2

SAMORIN I Samorin^{R} labelled on the phenanthridium molecule
SAMORIN II Samorin^{R} labelled on the benzamidine moeity

Table 2. Activity of the peaks detected on HPLC in the kidney and liver samples of the animals killed at different intervals

	Rt (min)	Liver % Act.	Kidney % Act.
SAMORIN I			
21 days p/t	4.45	9.6	0.0
	5.03	24.9	19.1
	8.11	34.3	26.3
	8.75	12.1	25.6
Total Act.		90.9	71.0
SAMORIN II			
47 days p/t	2.06	nd	16.5
	3.20	22.1	2.1
	4.45	nd	0.0
	8.11	nd	nd
	8.75	nd	nd
Total act.		22.1	18.6
SAMORIN I			
60 days p/t	2.06	0.0	nd
	2.46	4.3	5.1
	3.20	0.0	15.2
	4.45	22.4	6.0
	8.11	38.5	nd
	8.75	nd	nd
Total act.		63.2	26.3
SAMORIN II			
60 days p/t	2.06	nd	19.6
(average of	2.46	8.4	15.2
2 animals)	3.20	15.9	16.1
	4.45	0.0	0.0
	8.11	0.0	0.0
	8.75	nd	nd
Total act.		24.3	50.9

nd = no data

both tissues. However, the amount of the other five unidentified peaks were the same in both tissues. The presence of activity in the two peaks (rt. 2.46 and rt. 3.20) from samples treated with the two types of Samorin, indicates that some of the drug is converted directly into its derivatives. Furthermore, it seems that ISMM is stored in both tissues but it is not excreted as an intact drug but as products which are derivatives of homidium and benzamidine.

From these preliminary results it is evident that homidium has a great role to play in the prophylactic properties of Samorin. It would be interesting to determine what kind of products are present in plasma that seem to have trypanocidal activity at levels equivalent to 1 ng/ml of ISMM, for which trypanocidal activity was reported by Kratzer et al. (unpublished data). Systematic studies of the bile, urine, blood, liver and kidney of Samorin treated cattle are needed for further investigation of the metabolites of ISMM.

Table 3. Peaks detected on HPLC from tissue samples of the animals treated with the two types of C^{14}-Samorin^{R}

Rt. (min)	SAMORIN I	SAMORIN II
2.06	-ve	+ve
2.46	+ve	+ve
3.20	+ve	+ve
4.45	+ve	-ve
5.03	+ve	-ve (homidium)
8.11	+ve	-ve
8.75	+ve	+ve (Samorin^{R})

+ve = peak with activity
-ve = peak without activity

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PRELIMINARY STUDIES ON THE UPTAKE AND EFFLUX OF RADIOLABELLED DRUGS BY SUSCEPTIBLE AND DRUG-RESISTANT *Trypanosoma brucei brucei*

(Summary)

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^{14}C-labelled diminazene aceturate or isometamidium chloride has been added to bloodstream form cultures of drug-susceptible and drug-resistant Trypanosoma brucei brucei at concentrations similar to those occurring in animals after drug-treatment. The uptake of the labelled drugs by the trypanosome stocks or clones was followed for 24 hrs in vitro. When isometamidium chloride was used, no differences were detected in the uptake by one sensitive and two resistant stocks/clones. Furthermore, the rate of efflux of drug was similar between sensitive and resistant trypanosomes. When diminazene aceturate was used, the uptake of drug was about 4 fold higher in two sensitive clones when compared with two drug-resistant clones.

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USE OF RADIOLABELLED TRYPANOCIDES FOR SENSITIVITY SCREENING

(Summary)

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Large body of information has been accumulated especially during the last twenty years on the physiology, biochemistry and pathology of salivarian trypanosomes and particularly of tsetse transmitted African trypanosomes.

Very important scientific results have been obtained on the broad antigenic diversity of trypanosomes and on how they undergo antigenic variation to evade the hosts' immune response. However, this information has not yet produced any consistent and tangible evidence on how trypanosomiasis could be effectively and economically controlled, and the conventional methods of trypanosomiasis control, i.e. the use of trypanocides against the parasite and insecticide application against the vector are still the two major tools, with all connected constraints available to deal with the problem.

One of the major constraints of trypanosomiasis control with trypanocides is the development by the trypanosomes of resistance against all known drugs, which unfortunately are few in number. This problem is confounded further by the vague knowledge of whether trypanosomes develop actual resistance to the trypanocide, or avoid trypanocide action by slotting to inaccessible sites (cryptic sites) in the body. It is also not unlikely that trypanosomes could revert to a form or stage less susceptible to trypanocidal attack in the host as some irregular forms have been observed in experimental mice tissues (liver, brain, etc.) subjected to trypanocidal action for drug sensitivity tests.

The use of radio-labelled trypanocides could be of immense practical significance in unravelling these mysteries and in determining (i) whether trypanosomes develop actual resistance to trypanocidal drugs or avoid/accidentally escape trypanocidal effect by occupying an inaccessable position in the host and (ii) if there are forms or stages of the parasite in the host which are less amenable to drug action.

These and related issues are detailed in the main text to be presented on the conference.

Part III

TSETSE FLY BIOLOGY, ECOLOGY, VECTORIAL CAPACITY

FAT AND HAEMATIN CONTENTS OF MALE TSETSE FLIES *Glossina pallidipes* AND *G. m. morsitans* (DIPTERA: GLOSSINIDAE) CAUGHT IN ODOUR-BAITED TRAPS AND ARTIFICIAL REFUGES IN ZIMBABWE

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Abstract

Male G. m. morsitans and G. pallidipes caught in artificial refuges had 27 - 31% more fat than those caught in traps; mean haematin levels were 6.8 - 7.7 times higher, but haematin-specific fat levels did not differ between capture methods. Male G. pallidipes with haematin levels >150 ug, which had fed <8 h previously, contained 3.2 mg fat - only 15% less than the highest observed levels. A differential equation model for bloodmeal metabolism is developed; it removes 99% of the variance in fat levels of male G. m. morsitans fed and then starved in the laboratory and 79% of this variance for the G. pallidipes field data. The model predicts a mean feeding interval (T) of 53 h and fat levels of 3.1 mg in newly fed flies - close to the observed value of 3.2 mg for flies containing more than 150 ug haematin. Haematin frequency data, analysed as suggested in the literature, suggested T = 71 h, with a 60 h non-feeding phase, but fat levels predicted by simulation were 40% lower than observed. For constant feeding rates, fat levels were well simulated for T = 53 h, but death rates (>5%/day due to starvation alone) were impossibly high. An alternative model, based on known changes in activities related to feeding, suggests that feeding rates increase linearly during the trophic cycle. For T = 53 h the model gives good predictions of fat levels in G. pallidipes, with starvation rates <1%/day; it is suggested that a proportion of tsetse with high fat and haematin feed off mobile hosts early in the trophic cycle. Over-estimates of T result from the failure to consider these flies and not to errors in the assumed time scale, nor failure to catch high-fat flies which visit stationary traps.

Introduction

Tsetse flies, Glossina spp., feed only on blood, the energy content of which they store largely as fat. Residual blood meal and fat levels thus yield important information about a fly's nutritional state and Bursell (1961) pioneered attempts to interpret tsetse behaviour in terms of this information. The approach has since been developed by Randolph and Rogers (see references) who have advanced many new ideas regarding fly activity, feeding patterns, trappability and rates of fat utilization, relative to fat and haematin contents. Since the present study involves detailed discussion of their work it is important to understand the arguments advanced by these authors.

Randolph and Rogers (1978) drew attention to the similarity between the changes in percentage fat content with time (t) after feeding in laboratory flies (Fig. 1a) (Brady, 1975), and plots of percentage fat against log haematin for field-caught tsetse (Fig. 1b). Since log haematin declines linearly with t (Langley, 1966), Randolph and Rogers (1978) argue that such plots can be used to determine the time course of fat changes in the field, during the "hunger" or "trophic" cycle (Jackson, 1933; Brady, 1972). They concluded that "fat levels are partially responsible for triggering feeding behaviour" and that "where flies are able to feed relatively early in the hunger cycle, it is only those with below average fat levels that do so".

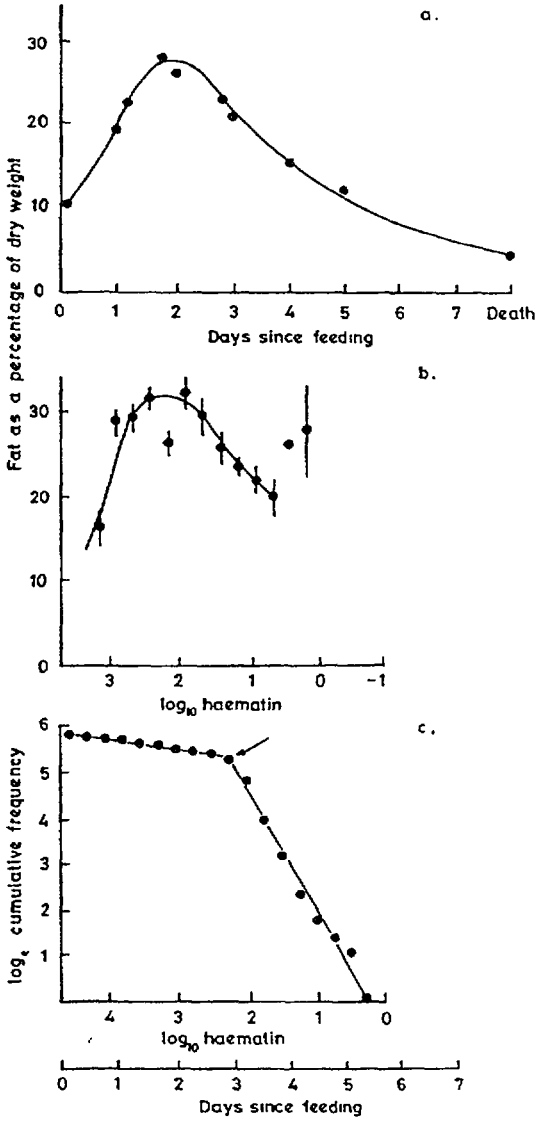

Fig. 1. a.) Changes in percentage fat levels of male G. m. morsitans during starvation in the laboratory; redrawn from Brady (1975).

b.) Percentage fat levels (± one standard error) of male G. m. morsitans at different levels of log haematin for samples caught at Matalawe, Tanzania; redrawn from Randolph & Rogers (1978).

c.) Cumulative frequency of haematin contents of male G. m. centralis Machado caught in Zambia. The arrow marks the boundary between the presumed feeding and non-feeding phases.

Rogers (1984) argued that an apparent discontinuity (Fig. 1c) in frequency plots of haematin content, for various tsetse species, marked the boundary between feeding and non-feeding phases, and that the linearity to the right of the discontinuity indicated a constant feeding rate thereafter. He estimated this rate from the slope of the graph, and the mean time taken to find and feed on a host from its inverse. He concluded that most tsetse feed late in the trophic cycle and that once they start feeding they find a host rapidly (generally in less than one day). Assuming that the rate of haematin excretion in G. m. centralis is the same as in G. m. morsitans (Langley, 1966) the breakpoint in Fig. 1c implies that the non-feeding phase lasts c. 69 h and that the flies find and feed on a host on average c. 9 h later - c. 78 h after the last feed. This is consistent with estimates of a mean inter-feed interval (T) of 3-4 days, from mark-recapture studies (Jackson, 1954; Glasgow, 1961; Rogers, 1977), and three days from physiological studies on female G. pallidipes in the field (Randolph et al., 1990).

By contrast, Langley & Stafford (1990) estimate that females of both G. m. morsitans and G. pallidipes must take more than three blood meals to produce a larva every nine days and that, therefore, T must be less than three days. Langley & Wall (1990) similarly conclude that T has been over-estimating and suggest that the methodologies developed by Randolph and Rogers are inappropriate. In this study we apply Randolph an Rogers' techniques and agree that they may well be over-estimated T, but not for the reasons cited by Langley and his co-workers; in general we cannot see any justification for rejecting Randolph and Rogers' methodology.

Previous studies have been hampered by the paucity of recently fed flies in the samples analysed. We have attempted to overcome the problem by collecting tsetse from artificial refuges (Vale, 1971) and are able to apply the techniques discussed above to samples of tsetse with a wider range of nutritional states.

Materials and Methods

Male G. m. morsitans Westwood and G. pallidipes Austen were captured at Rekomitjie Research Station, Zambezi Valley, Zimbabwe, in the study area described by Phelps & Vale (1978). Tsetse were sampled and pooled, within species, from six "box" type artificial refuges (Vale, 1971). Tsetse were free to come and go from the refuges at any time before the sample was taken, at between 1330 and 1430 h on each sampling day, but only enter when the temperature exceeds c 32°C; catch size increases approximately linearly with temperature (Vale, 1971). Refuge samples were taken on 12 days between 13 and 27 September, and on 12 days between 10 and 30 October 1988. Tsetse were also captured from an odour-baited epsilon trap (Hargrove & Langley, 1990) run between 0600 and 0830 h and between 1530 and 1800 h, on seven days between 14 and 27 September and on 12 days between 13 and 26 October. The trap was baited with acetone released at c. 200 mg/h and a mixture of 3-n-propyl phenol, 1-octen-3-ol and 4-methyl phenol in the w:w:w ratio 1:4:8, giving release rates of the three chemicals of c. 0.1, 0.4 and 0.8 mg/h respectively.

Flies for analysis were killed by transferring them, within 30 min of the end of the sampling period, to a freezer at -10°C and were kept in the dark at -10° - +2°C until they were processed, generally within 24 h and always within 72 h. The head, wings and legs of each fly were excised; the carcass and head were dried over calcium chloride, placed in labelled gelatin capsules and sent to the Tsetse Research Laboratory, Langford, Bristol, for fat, haematin and pteridine analysis (Langley et al, 1990; Lehane & Hargrove, 1989). The length and fray (Jackson, 1946) of the excised wings were assessed and analysis restricted to flies considered fully mature on the basis of pteridine content, wing fray and residual dry weight.

Results

Fat and haematin contents

The mean fat levels for G. m. morsitans and G. pallidipes from refuges were 1.9 and 3.3 mg respectively, 27 and 31% higher than the means of 1.5 and 2.5 mg from traps. Mean haematin levels, at 20 and 16 ug were 6.8 and 7.7 times the trap values of 2.6 and 2.4 ug respectively. The distributions differed significantly between methods (Figs. 2 and 3; $P<0.05$ for G. m. morsitans fat; $P<0.0001$ for all others, X^2). The refuges contained a proportion of flies with bright red abdomens swollen with blood - indicating that they had taken a large meal within about the previous 8h. Using the molecular weights of haemoglobin (68,000) and haematin (633 X 4 = 2532) and an estimate of 10% haemoglobin per wet weight of blood (P. A. Langley, pers. comm.), one unit of haematin residue is equivalent to a meal size of (68,000 X 10/2532 =) 268.6 units. The highest recorded haematin content for G. m. morsitans was 0.253 mg and for G. pallidipes 0.313 mg - equivalent to bloodmeals of 68 and 84 mg respectively, which are of the same order as the maximum blood-meal sizes of 49.9 and 92.4 mg measured for these flies at Rekomitjie (Taylor, 1976).

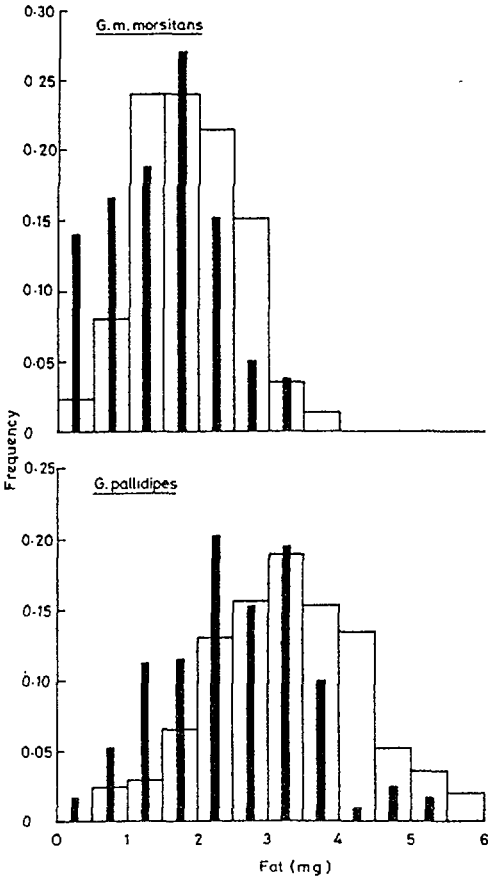

Fig. 2.

Fat contents of male tsetse sampled from traps (solid bars) and refuges (outlined histograms).

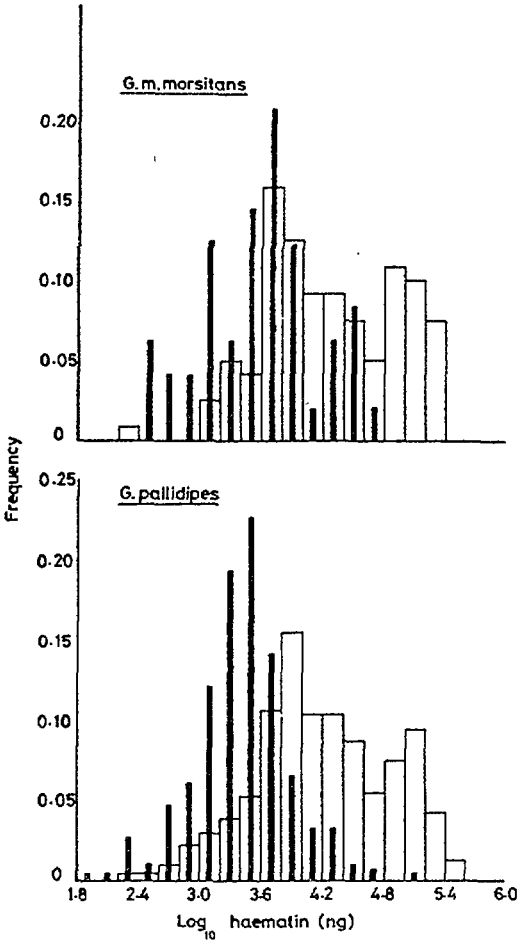

Fig. 3.

Log haematin contents of male tsetse sampled from traps (solid bars) and refuges (outlined histograms).

Fat levels at different levels of log haematin

Despite the differences in fat and haematin levels between tsetse taken from traps and from refuges, the 95% confidence intervals indicate no significant difference between sampling methods for mean fat at different levels of haematin (Fig. 4; results similar for G. m. morsitans) and these data were therefore pooled. The graphs of percentage fat against log haematin (Fig. 5) were of the form familiar from Randolph & Rogers' (1978, 1981) field studies, with fat apparently at low levels for some time after feeding. But when fat was plotted as the uncorrected variable, a different picture emerged. For G. m. morsitans only the most recently fed flies showed reduced mean fat contents (Fig. 5). For G. pallidipes the mean fat content even of flies with the highest haematin values was within 15% of the maximum found at any time during the trophic cycle. Thus, while the results for G. m. morsitans are ambiguous, those for G. pallidipes contradict the idea of tsetse feeding late in the trophic cycle when fat levels are low (see Introduction). The difference between the graphs of absolute and of percentage fat plotted against log haematin is not peculiar to this study; for G. m. morsitans caught using various mobile sampling devices in Tanzania (Randolph and Rogers, 1978) the same discrepancy exists (Fig. 6). Further examples from this and other work (Randolph & Rogers, 1981) show similar patterns and raise questions regarding the probity of expressing fat as a percentage of dry weight.

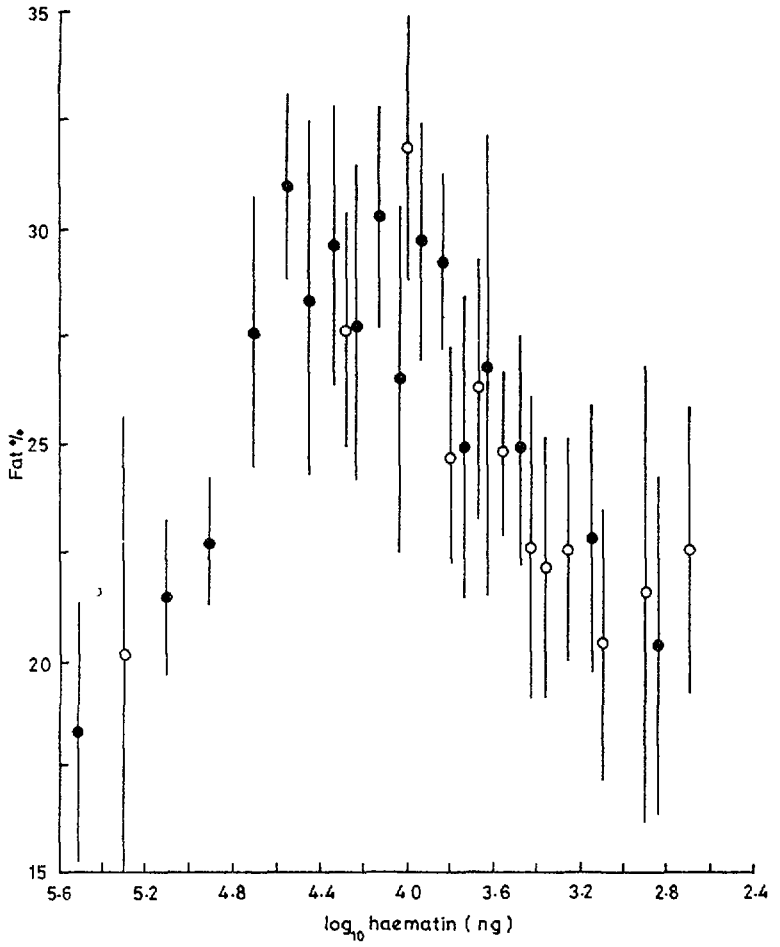

Fig. 4. Fat content (as a percentage of residual dry weight) at different levels of log haematin for male G. pallidipes sampled from traps (circles) and refuges (dots). Vertical bars indicate 95% confidence limits.

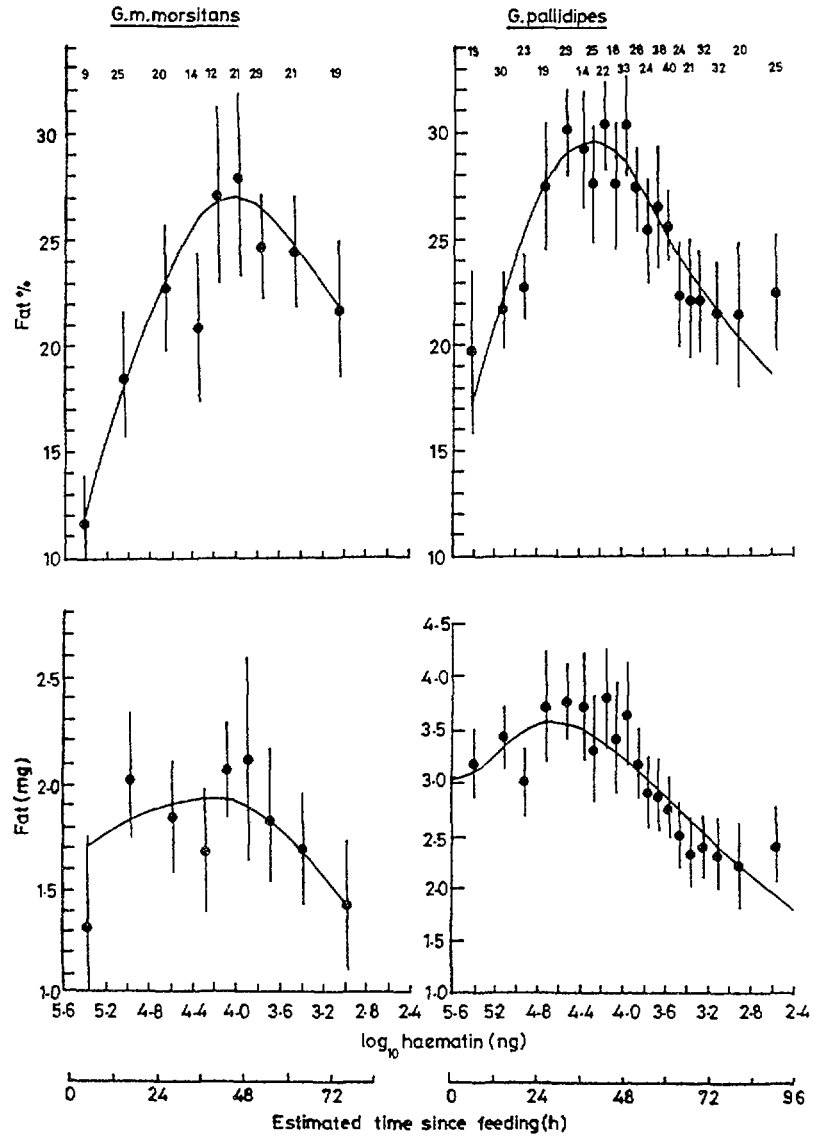

Fig. 5. Fat content at different levels of log haematin for male tsetse. Trap and refuge samples pooled and plotted as a percentage of residual dry weight (upper graphs) or uncorrected (lower). Vertical bars indicate 95% confidence limits; inset numbers are sample sizes.

Since (i) the ingestion of a full blood meal doubles the dry weight (Fig. 7) and (ii) the ingested blood itself contains less than 1% fat, there is a negligible increase in the fly's absolute fat content immediately after feeding. The percentage fat content remains low for some time, regardless of its absolute level (Fig. 5). In laboratory studies, when flies were fed following enforced starvation, whether absolute or percentage values were used made little difference (Brady, 1975); either way, flies which had just fed had low fat contents - the percentage plot was preferred since it reduced variance due to differences in fly size. For field samples, however, plotting percent fat against log haematin gives the impression that flies have low fat levels immediately after feeding and, by implication, feed on average only when fat falls to this level late in the trophic cycle. This seems to be false, at least for G. pallidipes, in this study (Fig. 5).

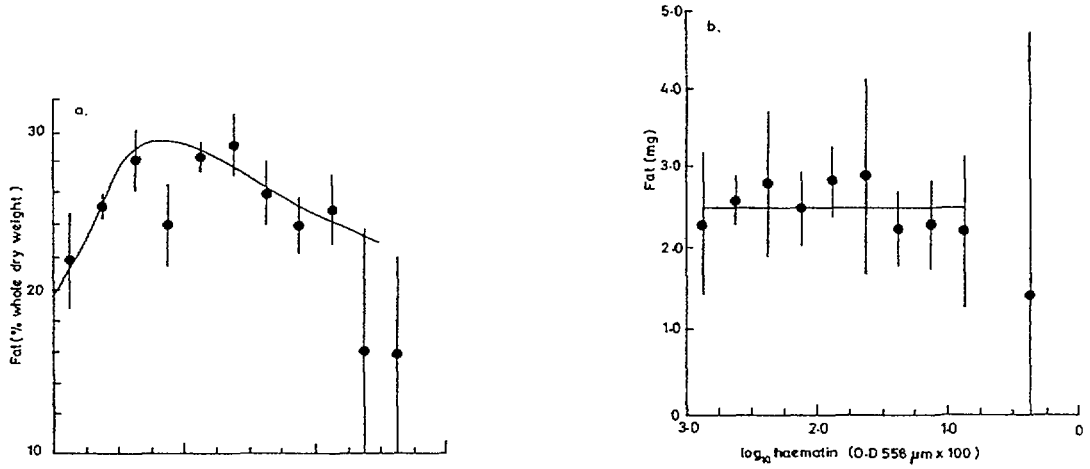

Fig. 6. Fat content at different levels of log haematin for male G. m. morsitans. Redrawn from Randolph & Rogers (1978). Hand net catches from Mau Mau, Tanzania. Upper graph: fat as a percentage of residual dry weight ± one standard error; line as fitted by authors. Lower graph: fat uncorrected; vertical bars indicate estimated 95% confidence interval. Fitted line at level of weighted mean fat over range shown. One mean, based on two flies, has been omitted.

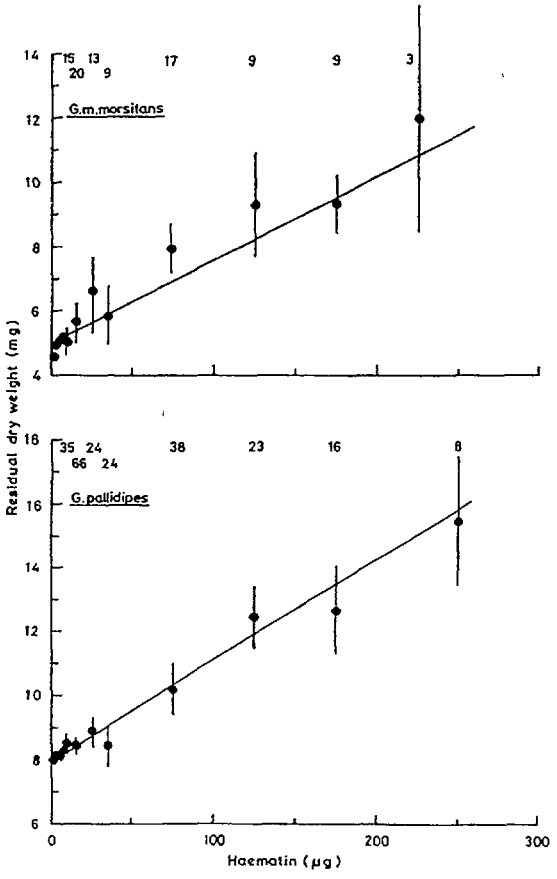

Fig. 7. Residual dry weight of male tsetse at different levels of haematin. Vertical bars are 95% confidence limits for means calculated on sample sizes shown at the top of each graph. Sample sizes for the four lowest haematin groupings were 30, 19, 24 and 12 for G. m. morsitans and 116, 94, 58 and 37 for G. pallidipes; 95% confidence intervals for the associated means ranged from 0.39 to 0.47 and from 0.10 to 0.26 for the two species respectively. Lines drawn from regression equations fitted to all data.

The variance in fat contents due to differences in fly size was assessed by performing multiple linear regressions of fat on log haematin and wing length, over semi-linear portions of Fig. 5. For G. m. morsitans no effect of wing-length on fat content could be demonstrated, and for G. pallidipes the effect amounted, at most, to an increase of about 1 mg of fat for each 1 mm increase in wing length. The variation in wing length was also small; 94% lay within 0.35 mm of the mean. When the means in Fig. 5 were recalculated, after first correcting the fat contents to that of a fly with the average wing length for the G. pallidipes used in the study, the average absolute change in the means was only 1.7%. The effect on the standard deviation was also small and not always beneficial; for 12 of the 21 means the standard deviation was decreased (by an average of 4.3%), for the other 9 it showed a small increase (2.7% on average). Correcting for size was thus unnecessary in this study; expressing fat as a percentage of dry weight is inappropriate and misleading in any field study.

Haematin frequencies

When log cumulative frequencies of haematin were plotted as suggested by Rogers (1984) it was perfectly possible to imagine a discontinuity in the graphs (Fig. 8; inset). But the data are also well described by the single, continuous, "log survivorship" function

$$\Phi(t) = e^{(-u(t).t)} \qquad (1)$$

where

$$u(t) = m_1 e^{(m_2 t)} \qquad (2)$$

is the instantaneous "mortality" rate - in this case the rate of recruitment into the sample. The rate increases exponentially with t for both capture systems and for both species (Fig. 9, inset); it is initially higher for refuges but increases more rapidly for traps. The probability that a fly is recruited in any interval (t_1,t_2) is given by $1 - (\Phi(t_2)/\Phi(t_1))$ and this increases approximately exponentially with decreasing values of log haematin (increasing t) and then levels off as it approaches probability one (Fig. 9).

Whatever the real nature of the recruitment function, two arguments suggest that the frequency data provide no basis for a sharp separation between feeding and non-feeding phases. (i) The discontinuities for G. pallidipes (Fig. 8) occur at different times for refuges and traps, whereas the timing should not differ between sampling systems (Rogers, 1984; Fig. 2b). (ii) The recruitment rate is determined by the product of two functions: a) the number of tsetse actually present in the population in each log haematin category (determined by the feeding pattern) and b) the probability of capture for a fly in that category (determined by the bias of the sampling system). Neither function can be calculated from graphs like Fig. 8, unless the other is known. Rogers (1984) assumed that the discontinuity was due to the onset of feeding, and that the linear portion of the graph to the right of this discontinuity was evidence in favour of a constant feeding rate (r) given by the slope of the graph. Both features could be due, in part at least, to changes in capture probability.

When applied to the G. pallidipes data the method leads to a discrepancy between the observed and predicted fat levels of flies which have just fed. For trap caught flies, the discontinuity (the nominal start of the feeding phase) occurs at log haematin c. 3.6, and the slope, r, of the linear portion to the right of this is 2.86. Thus $1/r = 0.35$ and the mean time to feed is estimated to occur at log haematin levels of $3.6 - 0.35 = 3.25$ and fat levels of c. 2.3 mg, 39% less than the 3.2 mg found in recently fed flies (Fig. 5).

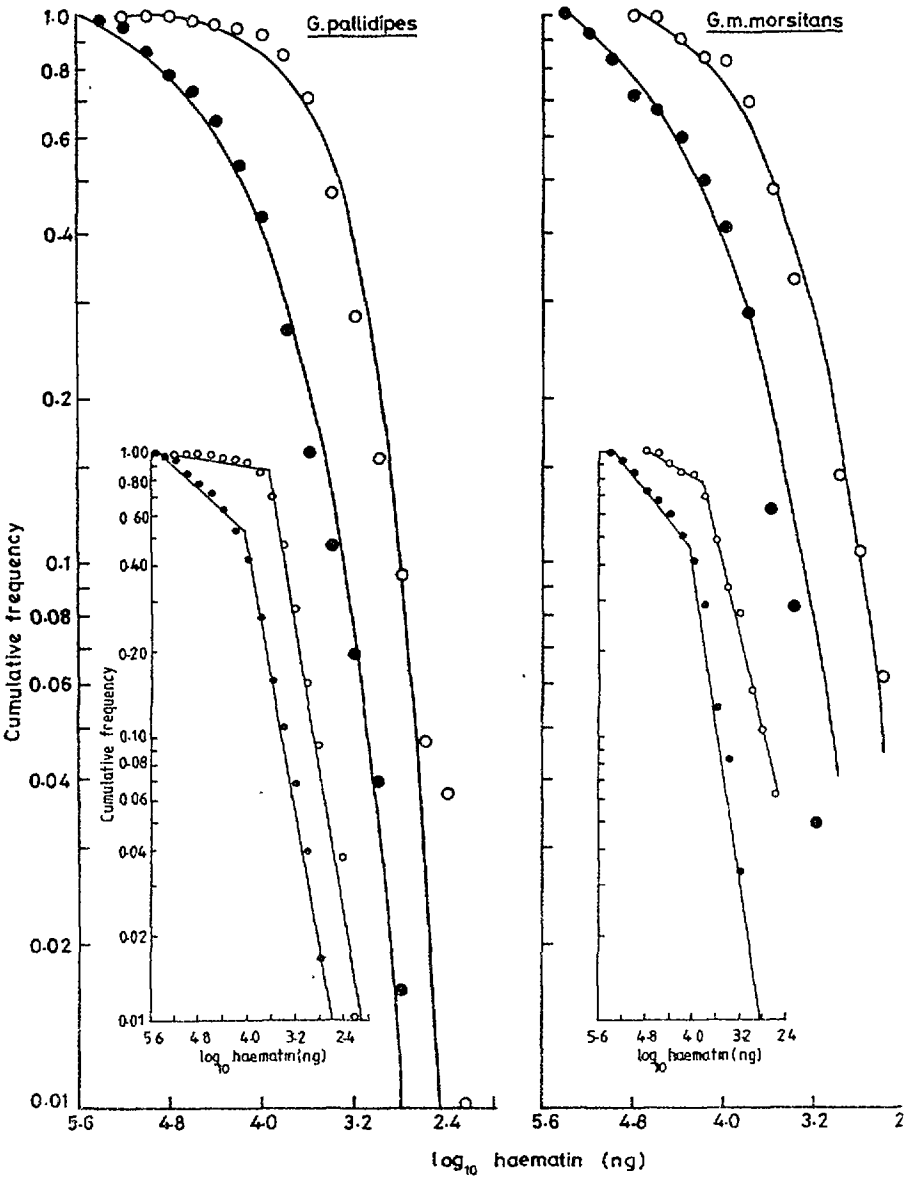

Fig. 8. Log cumulative frequency of haematin contents of male G. pallidipes (left) and G. m. morsitans caught from refuges (dots) and traps (circles). Fitted function given by equation (1) in text. Means and standard errors for parameters shown in Table 1. Inset: Lines fitted by eye assuming two linear functions with a discontinuity in the slopes.

The fat-haematin graph for G. pallidipes thus indicates that these flies feed earlier in the trophic cycle than estimated from the analysis of haematin frequencies. In an attempt to be more precise about the magnitude of the difference between the estimates, we developed the following model.

A new model for the metabolism of the blood-meal by tsetse

Bursell's (1963) data on the change in fat content with time (t) after feeding suggested the possibility that blood-meal metabolism might be regarded as a series of first order reactions (Fig. 10), with rates constants k_1 and k_2, for a fly with fat content F_0 and digestive product D_0 at the time it ingests a blood-meal of size B_0. From this model, the levels of B_t,

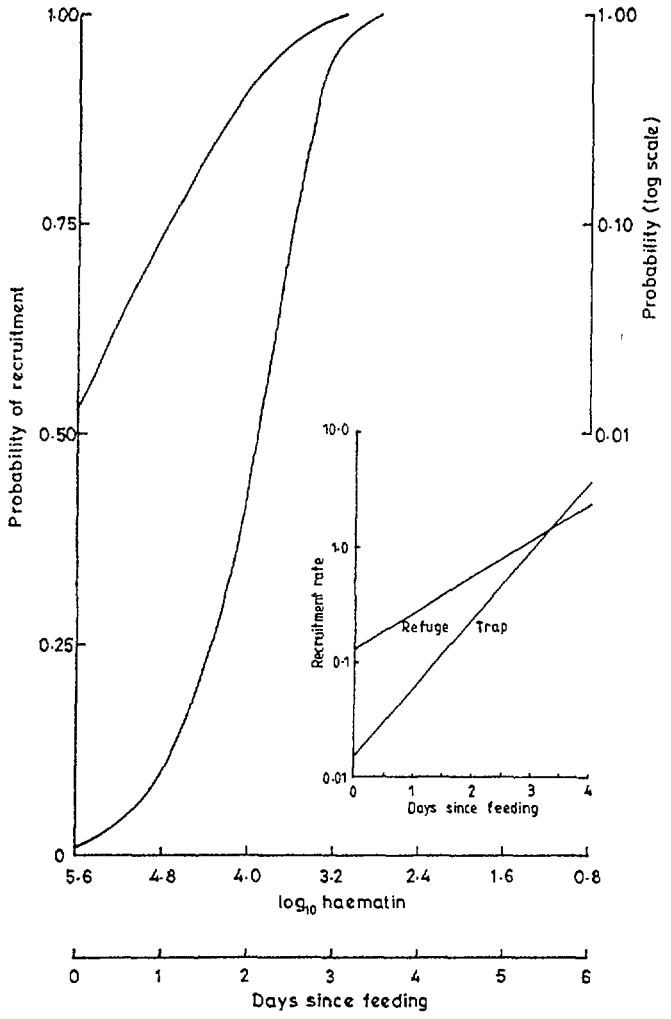

Fig. 9. The probability of recruitment (and the log of this function) of male G. pallidipes by traps as a function of time after last feeding - implied by the recruitment rate given in the inset. Inset: log recruitment rate to traps and artificial refuges for male G. pallidipes during the trophic cycle. Plotted function is given by equation (2) with parameter values given in Table 1.

D_t and F_t can be predicted at time t after ingestion by solving a set of first order differential equations (see Appendix). Fat levels at t are given by

$$F_t = (F_0 + K^2B_0)e^{-k_2t} - B_0K(K + k_1t))e^{-k_1t} \qquad (3)$$

where $K = k_1/(k_1 - k_2)$ and $D_0 = 0$ by assumption.

When equation (3) is fitted to Bursell's (1963) data by non-linear regression it accounts for 99% of the variance in the estimated fat contents (Fig. 10). The standard deviations of the parameters k_1, k_2 and B_0 are 5-7% of the estimates (Table 1); F_0 is predicted with less precision, but still within 50 ug of the true value. (The rate constants between B_t and D_t, and between D_t and F_t, were originally assumed distinct, but preliminary work shows no significant difference between the two parameter estimates).

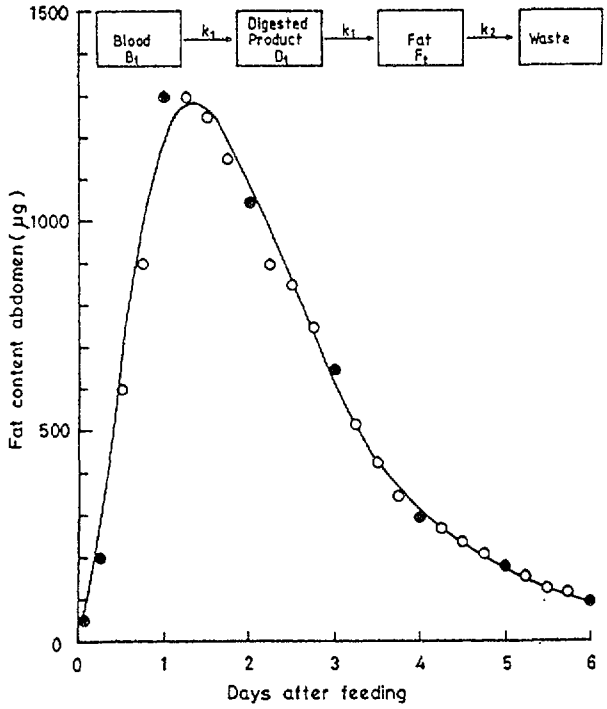

Fig. 10.

Bursell's (1963) measurements (dots) of the changes in the abdominal fat content of male G. m. morsitans which were starved, then fed and kept at 30°C until death. Circles are points interpolated on Bursell's (1963) eye-fitted curve. Fitted function computed according to equation (3) using the parameter estimates given in Table 2. Inset: first order model for the metabolism of the blood-meal in tsetse.

TABLE 1. Parameter estimates arising from the fit of the function $\Phi(t) = \exp(-m_1\exp(m_2 t).t)$ to the data shown in Fig. 8. Standard deviations shown in parentheses beneath each estimate.

Species	System	m_1	m_2
G. m. morsitans	Trap	0.150	1.055
		(0.023)	(0.102)
	Refuge	0.297	0.625
		(0.040)	(0.101)
G. pallidipes	Trap	0.016	1.711
		(0.004)	(0.136)
	Refuge	0.134	0.903
		(0.010)	(0.043)

The model also provides a good description of changes in the fat levels of field G. pallidipes (Table 2; Fig. 5). It was assumed that log haematin took a value of 5.6 for t = 0 and declined by 0.8 units per day thereafter - as for G. m. morsitans (Randolph & Rogers, 1978). The mean feeding interval (T) was estimated by inserting the estimated parameter values into equation (3) and solving for t corresponding to the point where fat fell to the level it held at the time of feeding. For G. pallidipes the estimated value is 53 h (log haematin 3.8), 19 h less than the 71 h (log haematin content 3.25, see above) estimated using the haematin frequency analysis. Both estimates of T depend on the assumed rate of haematin excretion but, since both can be expressed directly in terms of log haematin values, the discrepancy between them is independent of the chosen time scale.

The relatively scant results for G. m. morsitans make it impossible to obtain convergence when all four parameters in equation (3) are estimated, and application of the above analytical method to this species must await the collection of more data. The results for G. pallidipes call into question i) Rogers' (1984) strict division of the trophic cycle into a non-feeding phase followed by feeding at a high constant rate and ii) the estimates of T

TABLE 2. Parameter estimates resulting from fitting Equation (3), for changes in fat levels with time after feeding, to laboratory and field data for the tsetse flies G. m. morsitans and G. pallidipes. The asymptotic standard deviation for each parameter estimated is in parentheses beneath the estimate. Weighting by the inverse of the variance was applied for all the field data prior to analysis.

Key: G. m. morsitans (data estimated from Bursell's (1963) Fig. 1); G. pallidipes (data from present study, Fig. 5); n = number of estimates used in regression; k_1, k_2, F_0 and B_0 are the parameters for the model in Fig. 10; r^2 = fraction of variance removed by regression; T = estimate of mean feeding interval in hours.

Species	n	k_1	k_2	F_0	B_0	r^2	T
G. m. morsitans	25	0.0901	0.0295	-6	2543	0.99	-
		(0.0051)	(0.0020)	(37)	(135)		
G. pallidipes	21	0.0775	0.0150	3089	2889	0.79	53
		(0.0290)	(0.0060)	(409)	(1286)		

implicit both from that type of analysis and from the shapes of graphs of (percent) fat against log haematin (Randolph and Rogers, 1978). Rogers (1984) noted that his model was at variance with laboratory results indicating smooth changes in activity patterns during the trophic cycle. We develop below a model which attempts to provide a sensible link between laboratory and field results.

An alternative model for changes in feeding rates during the trophic cycle

The feeding process is regarded as a series of events occurring at instantaneous rates which may vary with t. We assume, for simplicity, that flies detect hosts and alight on them (Fig. 11) via one of two routes: A) inactive flies react visually to a passing host; B) spontaneously active flies detect a host using smell and sight. For each route the feeding rate, as with the overall rate of a chemical reaction, is determined by the minimum rate for that route; and the total feeding rate at any time is the sum of the two minima. We are not yet in a position to quantify the rates shown in Fig. 11, and the model is perforce simplified and speculative but, wherever possible, is tied to published data.

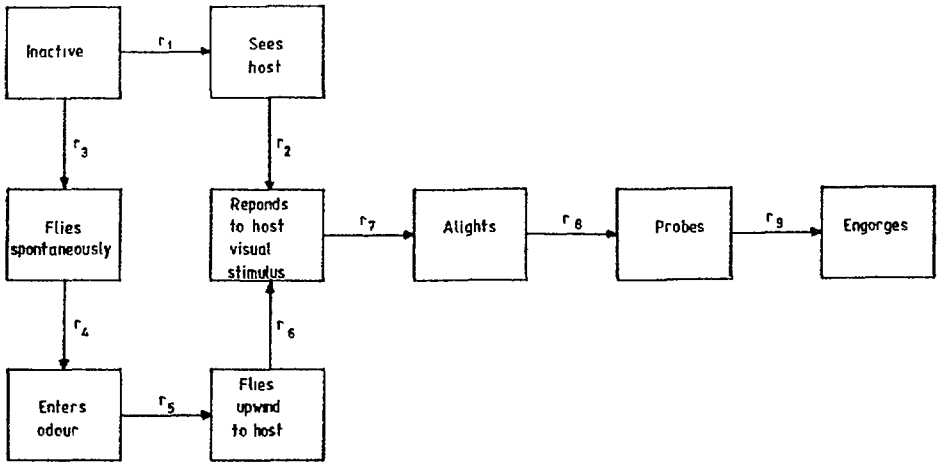

Fig. 11. The feeding process in tsetse modelled as a series of reactions leading to feeding off a mobile or a stationary bait.

In the laboratory, the rates of spontaneous activity (r_3) and of response to a mobile target (r_2) increase exponentially with t - at about the same rate but with the latter always higher (Brady, 1975). The rate (r_6) at which flying tsetse respond to a visual target is assumed identical to r_2. Probing rates (r_8) in the laboratory increase linearly with t (Brady, 1973) and in the field c. 90% of G. pallidipes landing on a stationary ox then probe, though only 35 - 40% of these engorge (Hargrove, 1976). We therefore assume that the rate of engorgement (r_9) is always lower than the probing rate; it is also assumed to increase linearly.

It seems reasonable that the rates at which flying tsetse detect odour plumes (r_4), and fly up them (r_5), are proportional to the fly's rate of spontaneous activity and thus increase exponentially at the same rate. We assume, similarly, that the alighting rate (r_7) increases exponentially at the same rate as r_2 (and hence r_3). Finally it seems reasonable to assume that the rate at which tsetse see hosts walking past their resting sites is independent of t. All rates involving the actual detection of hosts

depend on such factors as host density and visibility - in turn dependent on vegetation cover and season - and these factors will affect the limiting rates for each route in different ways.

The suggested patterns of rate changes are shown in Fig. 12a - where only the lowest of the exponential rates is shown for each route. We assume that r_2 is the minimum exponential rate for route A and r_3 for route B. Fig. 12b illustrates how the rate limiting step might change with $\underline{t}$ for each route. For recently fed flies the engorgement rate (r_9) is the minimum on both routes. Later, on route A, r_9 increases and surpasses the rate (r_1) at which tsetse see hosts walking past them; r_1 then becomes the limiting step and remains so for the remainder of route A (Fig. 12a). The rate of spontaneous activity (r_3) is limiting on route B except in the earliest and latest stages where the engorgement rate (r_9) is even lower.

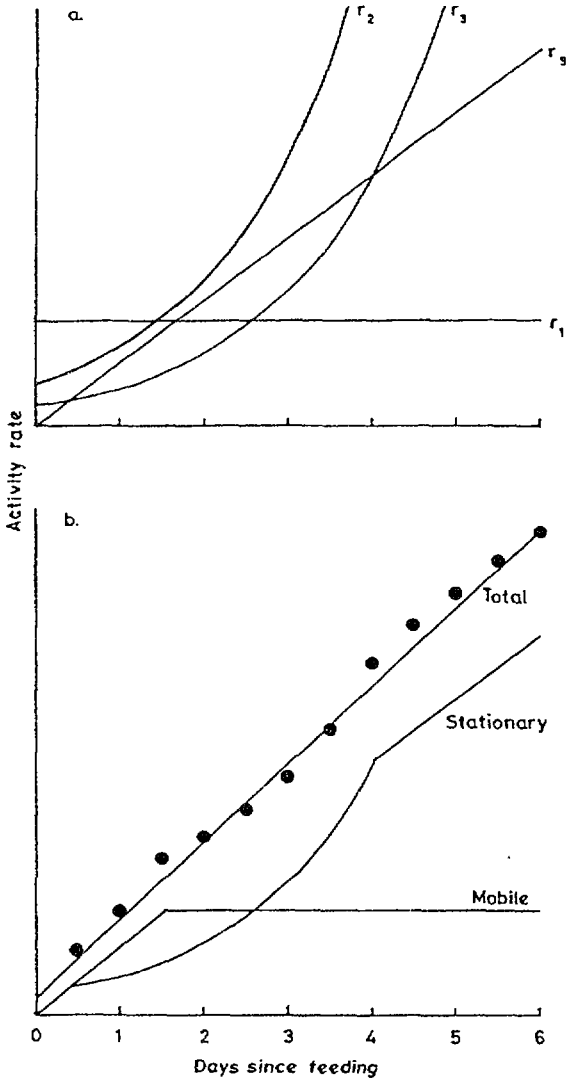

Fig. 12. a) The suggested changes in some of the reaction rates during the trophic cycle for the tsetse feeding model shown in Fig. 13.
b) The envelopes of the minima for the reaction rates r_1, r_2 and r_9 (mobile) and for r_3 and r_9 (stationary) and the sum of the two envelopes estimated at 12 h intervals (total).

The envelopes for the minima on the two routes, and their sums, are shown in Fig. 12b which indicates a shift from mobile to stationary baits during the trophic cycle, and a total feeding rate increasing approximately linearly with t. If the rate of this increase is k then the probability, $\Phi(t)$, that a fly has not fed by time t is

$$\Phi(t) = \exp(-k.t^2) \qquad (4)$$

and the probability that it has, is $1-\Phi(t)$. The probability that it feeds in time interval (t_1,t_2) is given by $1 - \exp(-k.t_2^{\ 2})/\exp(-k.t_1^{\ 2})$; these functions are illustrated in Fig 13 (cf. Fig. 9). We test below, using simulation, which of three feeding rate functions i) a constant feeding rate ii) a linearly increasing rate, and iii) a discontinuous function of the type suggested by Rogers (1984), produces the best fit to the G. pallidipes data.

As an aside, note that for recruitment to (stationary) odour-baited traps the individual rates should be as for route B until r_6 - i. e. until the flies arrive at the trap. If the rate of entry to the trap is always high (see Discussion) it is possible that the limiting factor is always the fly's rate of spontaneous activity. If so, the rate of recruitment to traps would increase exponentially with t - as seems reasonable from Fig. 9.

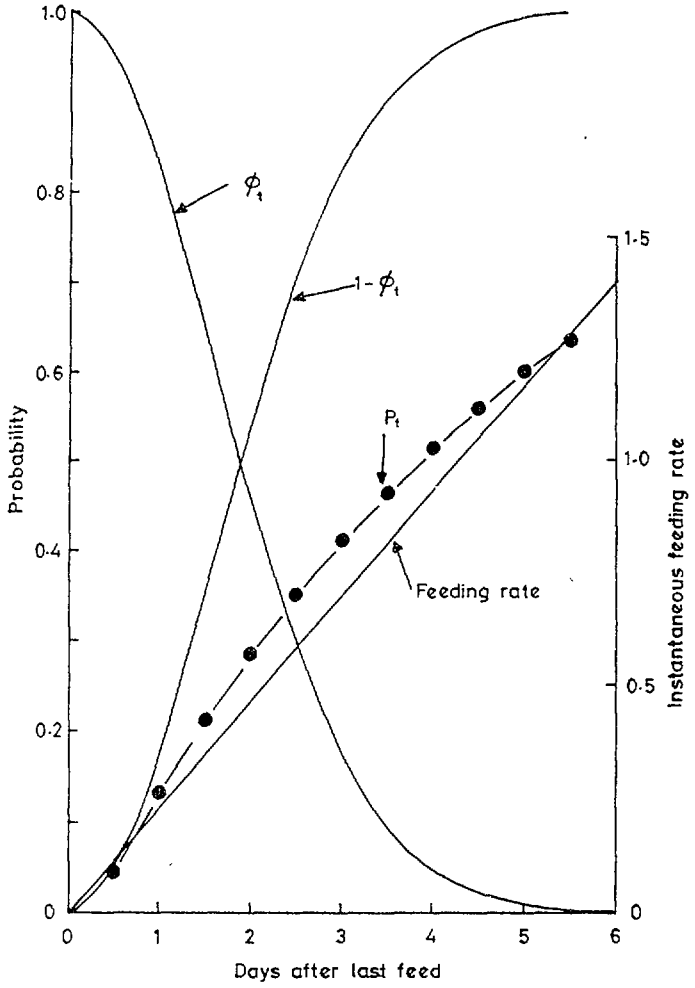

Fig. 13.

Feeding probabilities arising from a linear increase in the instantaneous feeding rate. The function $1-\Phi_t$ and its complement are the probabilities that a fly has, or has not, fed by time t. Pt is the probability that a fly feeds in successive 12 h periods.

Simulations of the feeding cycle and blood-meal digestion in tsetse

In each run individual "flies" started with predetermined levels of fat (F_0) and digestive product (D_0) and were given an input (B_0) equal to 2889 units of blood as estimated for G. pallidipes (Table 2). Using these inputs and the parameter values from Table 2 the levels of B_t, D_t and F_t present 12 h later were estimated from equation (3). The algorithm then tested whether the fly fed; a random number was chosen in the interval (0,1) and, if it was less than the stipulated probability (p) of feeding in that 12 h period, B_t was incremented by 2889 units; if not, no increment was made. In either event metabolite levels present after a further 12 h were calculated. Flies failing to feed for 12 consecutive 12 h periods were considered to have died of starvation and this fact was recorded. The procedure was carried on for a number of days sufficient for the fat levels for flies at different times post-feeding to stabilise. In preliminary runs, metabolite levels were checked after 30 or 40 days, using 1000 or 10 000 "flies", and for F_0 and D_0 either 0 or 3 mg, or a randomly chosen value in this interval. The resulting fat levels differed by <5% and, thereafter, runs were standardised to 40 days, using 10 000 "flies", with $F_0 = D_0 = 0$.

If G. pallidipes did not feed for 60 h after each meal, p had to be 0.9/12 h thereafter to give T = 71 h (see above); no flies died of starvation, but predicted fat levels were c. 40% lower than observed (Fig. 14c). If the non-feeding phase lasted only 48h and flies fed with p = 0.5 thereafter, so that again T = 71 h, fat levels were still markedly lower than predicted.

With p a constant 0.15 throughout, T = 53 h and fat levels were predicted tolerably well (Fig. 14d) - but 90% of the flies were judged to have died of starvation, at some point during the 40-day run, using the criterion of failing to feed for six consecutive days . This is equivalent to a 5.5% daily death rate, which could not be sustained by a non-decreasing population (Rogers, 1984; Hargrove, 1989). For T = 71 h (see above) p had to be reduced to 0.05, and 99.99% of the flies died of starvation in 40 days. (It was thus impossible to gauge fat levels). For p = 0.3, starvation rates were <1% per day, but then T = 36 h and fat levels were 60% higher than observed (Fig.14d).

When the feeding rate increased with t, at rate k = 0.3 (equation 4) so that T = 53h (Table 2), fat levels were adequately predicted, and the fit was even better for k = 0.25, when T = 58h (Fig. 14a). Starvation rates were <1%/day in both cases.

Rogers (1984) concluded, on general grounds, that simple models of feeding behaviour where tsetse "feed whenever they can" or show "several days of gradually increasing activity" would result in high death rates due to starvation. If a constant feeding rate is described by his first statement the above simulation supports his conclusion. But a linear increase in activity, which seems to be described by the second, did not lead to high starvation rates and it simulated observed results better than a constant feeding rate or one involving a prolonged non-feeding phase. Further simulations indicated that the function did not have to increase linearly; for example, an exponentially increasing rate produced good fits to the data. The important features seemed to be that i) T had to be in the region of 55h ii) the feeding rate should increase with t and iii) any non-feeding phase must be shorter than indicated by the analysis of haematin frequency data.

Although the model developed used t as the measure of the way in which fly behaviour changes, we realise that other physiological factors must be involved. Arguments adduced by Randolph & Rogers (1978) are consistent with the idea that, for given levels of haematin, flies with lower than average fat

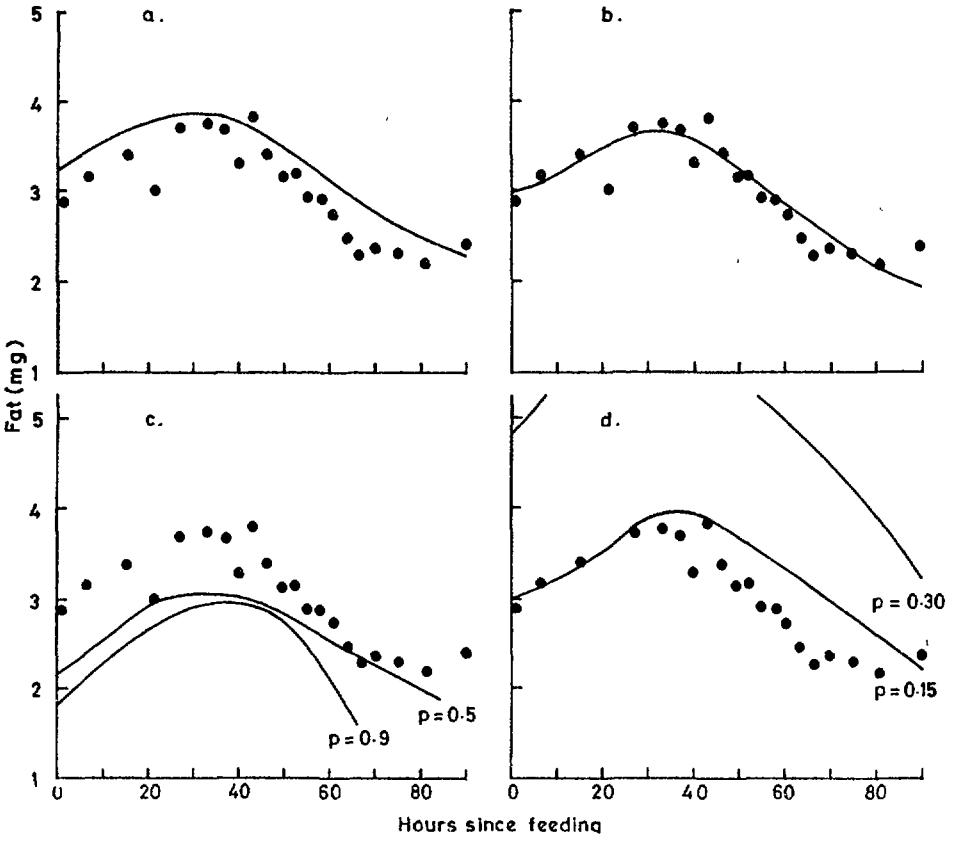

Fig. 14. Simulation of the fat content of male G. pallidipes (Fig. 5) using various feeding strategies and mean feeding intervals T. a). Feeding rate increases linearly; T = 53 h. b). As for a) but T = 58 h. c). Non-feeding phase lasting 60 h followed by feeding probability p = 0.9 per 12 h period, or lasting 48 h followed by feeding probability p = 0.5 per 12 h period. d). Constant feeding probabilities as shown.

have higher feeding rates. This is consistent with the finding that such flies are trapped with higher probability (Langley & Wall, 1990). For the model in Fig. 12 to make sense there must be some good reason for spontaneous activity to increase with t - otherwise an optimal foraging strategy could be as well achieved by being active at the beginning of the trophic cycle and inactive at the end. One possibility is that spontaneous activity is relatively inefficient early in the trophic cycle because of the large proportion of the aerodynamic power expended on lifting the proceeds of the last meal. This explanation is consistent with Brady's (1975) finding that the only physiological variables which correlated better than t, with the spontaneous activity of male G. m. morsitans, were abdominal weight and total fresh weight. This provides another potential link between laboratory and field work and one which might easily be tested in the laboratory, by increasing the fly's weight artificially (by the attachment of small lead weights, for instance) and observing the effect on spontaneous activity rates.

Estimations of sampling biases

The algorithm used above recorded the number of "flies" in each 12 h interval after feeding (Fig. 15, inset) and these figures can be used to calculate the sampling bias (or intensity) at each stage of the trophic cycle for traps and refuges, in the manner suggested by Rogers (1984). If there are n_j flies in time category j and $s_{i,j}$ of them are caught in a sample using method i, then the sampling intensity $S_{i,j} = s_{i,j}/n_j$. For the feeding rates in Fig. 13 the sampling intensity for traps increased in a rapid exponential way for most of the trophic cycle (Fig. 15); for refuges the changes were smaller, supporting Vale & Phelps (1978) contention that refuges give a less biased sample of tsetse than traps - in terms of the flies' nutritional state. The estimated biases, like those of Rogers (1984), depend on the assumed feeding rate function and, given the uncertainty surrounding the latter, cannot be used to estimate population size (Rogers, 1984) with any confidence at this stage.

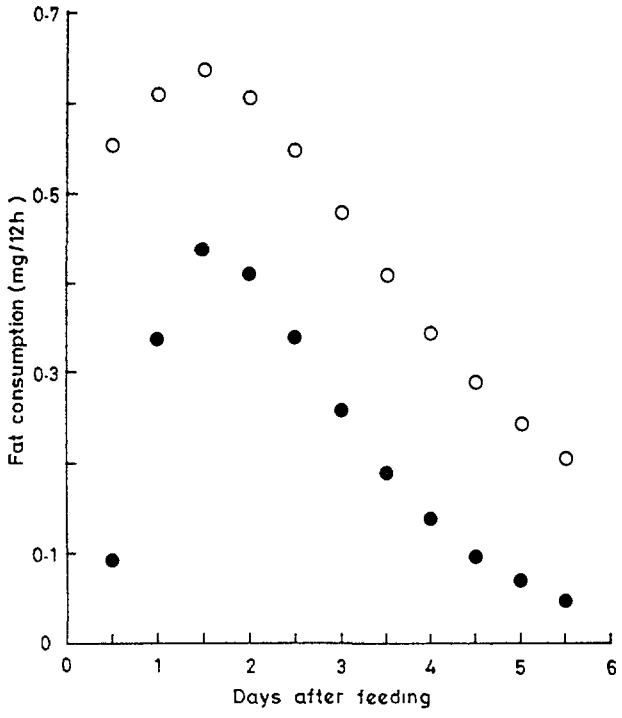

Fig. 15.

The sampling intensity for male G. pallidipes by odour-baited traps (dots) and artificial refuges (circles) at different times of the trophic cycle; calculated from the numbers estimated by simulation to be in each time interval and the number actually found (see Fig. 3). Feeding rates calculated from equation 4 with $k = 0.3$. Inset: the distribution of "flies" in each 12 h time category for two simulation runs where the total sample size was chosen so as to approximate the real sample from the refuges (solid bars) or traps (outlined histograms).

Rates of fat utilization and flight activity

The instantaneous rate of fat utilization is given, according to the model, by k_2 multiplied by the amount of fat present (Fig. 10). The total use in any period (t_1,t_2) is found by integrating equation (3) between these limits (see Appendix). The estimated rates of utilization for Bursell's (1963) laboratory G. m. morsitans, and field G. pallidipes from the present study, suggest that the rates peak about two days after feeding and then decrease by approximately 15% per day (Fig. 16). The high rates of fat utilization during the first two days after feeding are presumably associated with the heavy energetic cost of digestion and excretion, but it is strange that the rate should then decrease when all indications are that flight activity increases exponentially over this period (Brady, 1975). However, Brady's own data (see our Fig. 1a) indicate quite clearly that the rate of fat utilization does decrease over this period and Taylor (1977) found that oxygen consumption either decreased, or remained roughly constant, after digestion was complete.

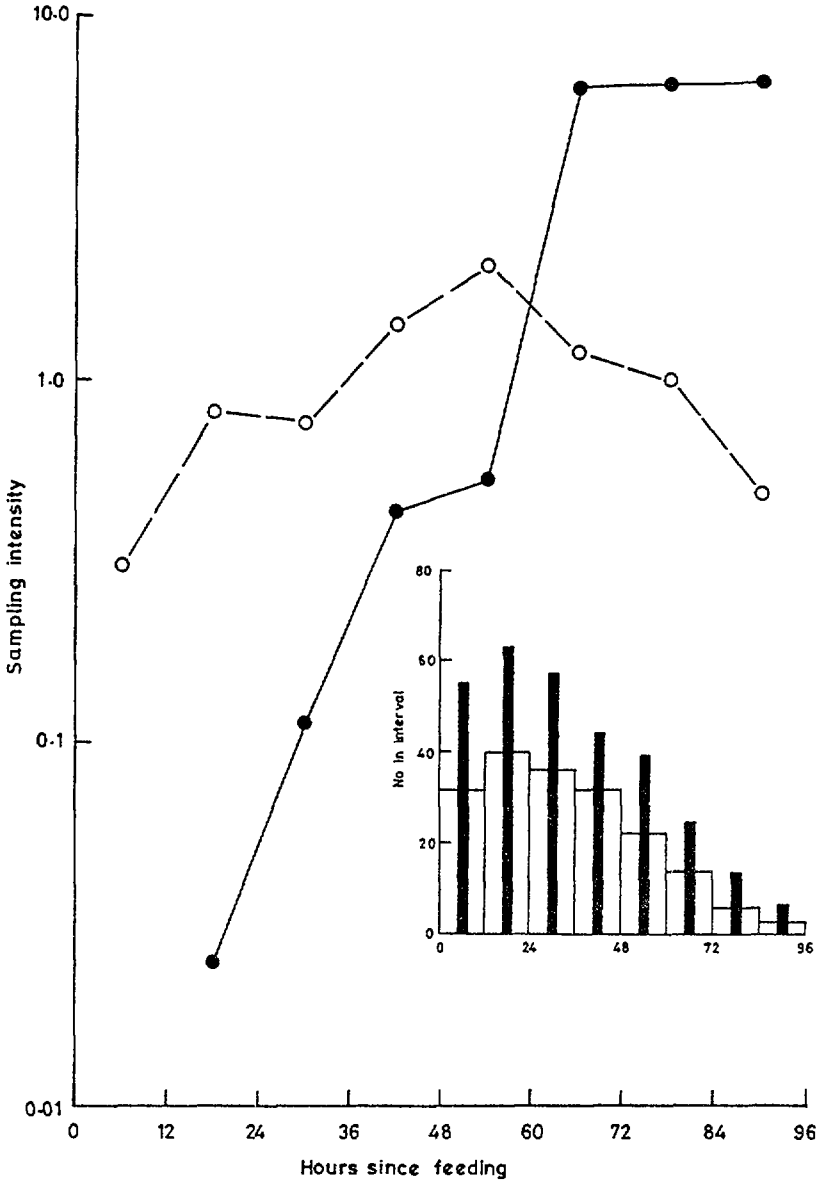

Fig. 16. Fat utilization per 12 h period at different times of the feeding cycle calculated for males of laboratory G. m. morsitans (dots) and field G. pallidipes assuming the model in Fig. 10 and the parameter values in Table 2.

Discussion

We conclude, as do Langley & Wall (1990), that T has been over-estimated in previous studies, but our reasons for doing so are quite different and we question some of their arguments in this regard. They found that many of the "well fed" tsetse which visit traps escape capture, and argued that many of them would feed on a host if present - and that failure to catch such flies has led to over-estimates of T. However, while the mean fat contents of trapped (1.46 mg) and untrapped (2.55 mg) male G. pallidipes were different, Langley & Wall (1990) failed to consider the absolute numbers of flies in each category. The data used were derived from an experiment conducted by Holloway, Hargrove & Vale (in preparation) which indicated that at most 15% of the male G. pallidipes visiting the trap escaped capture. The mean fat levels

for all flies visiting the trap may thus be estimated from ((85 X 1.46) + (15 X 2.55))/100 = 1.62 mg, only 11% higher than the value for trapped flies alone. Errors in estimating T would be thus be small even if all flies visiting traps would have fed.

In fact, the probability that a tsetse fly feeds on any particular visit to a host is remarkably low (Hargrove, 1976; Vale, 1977): for an ox or a kudu, generally less than 50% fed, and for buffalo, bushbuck, bushpig and warthog (all favoured natural hosts) the figures were 2-19%. The failure of traps to catch a small percentage of high-fat flies (which may be expected to feed with even lower probability than these average values) can therefore hardly be responsible for serious over-estimates of T.

Langley & Wall (1990) also argue that: "In view of the rapid rate of digestion of blood meals in the field .. and the large variation in haematin content at any given time after feeding it is doubtful whether haematin content measurements accurately reflect the time since the fly last fed, especially if flies feed again before all the haematin associated with the previous blood meal has been eliminated" and "therefore regressions of fat on haematin values to estimate rates of fat utilization should be treated cautiously". However, Langley & Wall's (1990) data for field flies (the only results of relevance here) do not support this argument. The highest value for haematin on each day is at least 0.4 log units lower than the lowest reading 24 h previously. The error is thus small and could not be responsible for mistaking, say, a three-day for a two-day feeding interval; still less could one estimate three days if the truth were 38 h, as they estimate from Bursell's (1980) data.

The remnant haematin from the previous meal is unimportant; after only 30 h it amounts to 10% of the original (assuming a drop of 0.8 log units per 24 h; see above) and this makes a negligible difference to the resulting time estimate. For example, if a fly has a haematin content of 400 ug just after feeding, and thus 40 ug 30 h later, just before it takes in another 400 ug, its new haematin content is 440 ug. The difference between the log values for 400 and 440 mug is 0.04; thus the error in estimating T would only be 1.2 h i.e. 4% of the real value of 30 h.

Despite the above remarks, there are other, apparently valid, reasons for thinking that female tsetse, at least, must feed more frequently than every three days. Langley & Stafford (1990) found, in the laboratory, that the best rates of larval production were achieved when G. m. morsitans females took four, and G. pallidipes five, blood-meals per reproductive cycle, and suggest this reflects the field situation. Randolph et al. (1990) found, similarly, that field G. pallidipes held in the laboratory required feeding every other day to ensure larval production; but they estimated that flies fed only once every three days in the field. They ascribed the discrepancy to the fact that "flies behave physiologically quite differently as soon as they are brought into the laboratory". While there is much evidence in favour of this idea, there is one point of physiological identity between field and laboratory flies - namely, that larvae of the same size from either source must have the same energy content. This requirement seems to indicate that G. m. morsitans and G. pallidipes in the field need more than three meals to produce a larva every nine days (Bursell & Taylor, 1980; Langley & Stafford, 1990) and must therefore feed more frequently than every three days.

The model summarised in Fig. 12 allows a new explanation of why T has been over-estimated. Tsetse only visit stationary baits late in the trophic cycle, but may feed off mobile baits before this time. There is no need to reject the use of haematin as a time-scale, nor the consequent estimates such

as those of Randolph et al. (1990) that female G. pallidipes which visited odour-baited traps did so, on average, three days after they last fed. As expected, such flies have low levels of fat and haematin, as with the male G. pallidipes from traps in the present study. But the presence of flies with more than 63 ug haematin, and more than 3 mg of fat, in refuge catches (Figs. 2 - 4) shows that stationary baits are failing to sample a crucial part of the population - flies which have fed while they still have relatively high fat levels. Stationary baits trap these flies with low probability for the obvious reason that, having recently fed and having high fresh weights and high levels of fat and haematin, they have low rates of spontaneous activity. On the other hand, according to our model, the probability is rather higher that a recently fed fly will feed off a host animal which walks past its resting site. The net result is that analysis of samples taken solely from stationary baits has tended to over-estimate T - not because flies which visit them are not caught (Langley & Wall, 1990) but because some nutritional classes, which might feed, do not visit traps at all.

Acceptance of this view allows the explanation of problems evident in a recent study by Randolph et al. (1990). Female G. pallidipes apparently added almost 4 mg of residual dry weight on days 8-9 of a 9-day reproductive cycle, but these were the days when the probability of feeding was apparently lowest. Since Randolph et al. also estimated that flies were feeding on average every three days, but hardly fed on day eight or nine, there should have been a high degree of synchrony, with a large proportion of the population feeding during the 24 h period following larval deposition. In fact, there was no such synchrony and no peak of feeding on day zero or one of the cycle.

The flies for this study were all caught from (stationary) traps. If a proportion of them were feeding off mobile baits earlier in the trophic cycle, T would be shorter and its variance greater than evident from the trap samples alone. This implies more than three meals per nine day reproductive cycle (Langley & Stafford, 1990) and could explain the apparent short-fall in residual dry weight and the lack of synchrony. The implicit, and testable, suggestion follows that females caught feeding on mobile and on stationary hosts should show different spectra of reproductive and nutritional states. In particular one expects an increased proportion of females in the later stages of pregnancy. Such flies exhibit low spontaneous activity in the laboratory (Brady & Gibson, 1983), consistent with a possible shift from stationary to mobile hosts in the field.

One can also predict that reliance on stationary baits causes the strongly cyclical nature of recapture rates (Rogers, 1977) in mark-recapture experiments, which have been used as evidence for the strict division between feeding and non-feeding phases (Rogers, 1984). The present analysis suggests that if tsetse were recaptured using mobile baits this cycle would be less clearly marked.

APPENDIX

The changes in metabolites in Fig. 1 are described by the following set of first order differential equations:

$$\frac{dB_t}{dt} = -k_1 B_t \tag{1}$$

$$\frac{dD_t}{dt} = k_1 B_t - k_1 D_t \tag{2}$$

$$\frac{dF_t}{dt} = k_1 D_t - k_2 F_t \tag{3}$$

with initial conditions $B_t = B_0$, $D_t = D_0$, $F_t = F_0$, which have solutions

$$B_t = B_0 e^{-k_1 t} \tag{4}$$

$$D_t = (D_0 + k_1 B_0 t) e^{-k_1 t} \tag{5}$$

$$F_t = (F_0 + KD_0 + K^2 B_0) e^{-k_2 t} - K(D_0 + B_0(K + k_1 t)) e^{-k_1 t} \tag{6}$$

where $K = k_1/(k_1 - k_2)$

For present purposes we assume $D_0 = 0$, in which case,

$$F_t = (F_0 + K^2 B_0) e^{-k_2 t} - B_0 K(K + k_1 t)) e^{-k_1 t} \tag{7}$$

The rate of fat utilization at any time t is, by assumption, $k_2 F_t$ and the amount used in any time interval (t_1, t_2) is found by evaluating the integral

$$\int k_2 F_t = [(k_2/k_1)(C + KB_0 + k_1 KB_0 t)) e^{-k_1 t} - (F_0 + C) e^{-k_2 t}] \tag{8}$$

(where $C = K^2 B_0$)

between the two limits.

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STUDY OF THE VECTORIAL CAPACITY OF *Glossina palpalis palpalis* RELATED TO ITS DIGESTIVE PHYSIOLOGY AND REARING CONDITIONS

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Abstract

The tsetse midgut environment constitutes the early barrier in the development of the parasite in his insect host. Factors such as trypanolysins, midgut lectins and agglutinis have been shown by various authors to influence the establishment of a procyclic trypanosome population. So far, the digestive process has not been considered as a possible factor affecting the survival and differentiation of ingested trypanosomes.

This paper deals with our investigations on the digestive physiology of Glossina palpalis palpalis. The course of the digestive cycle, the purification and partical characterization of midgut trypsin, the major proteolytic enzyme, are described. The possible interaction of the digestive activity and trypanosome development is discussed.

Introduction

Over recent years many studies have been focused on physiological and biochemical factors underlying the tsetse fly-trypanosome association. This basic knowledge is essential for a better understanding of the epidemiology of trypanosomiasis and could possibly contribute to the improvement of existing tsetse/trypanosomiasis control techniques.

Trypanosomes are ingested by the tsetse with a bloodmeal taken from an infected host. The midgut environment constitutes the early barrier in the development of the parasite in his insect host. Here trypanosomes have to adapt and transform to the procyclic form. The presence of trypanolysins and midgut lectins/agglutinins have been shown by various authors to influence the establishment of a procyclic trypanosome population.

Studies on other hematophagous insect vectors such as Phlebotomus and Aedes suggests a possible determining role of digestive enzymes in the development of their respective protozoan parasites. Hitherto, the digestive process in the tsetse midgut has not been considered as a possible factor affecting the survival and differentiation of ingested trypanosomes.

This report deals with our study on the midgut trypsin of Glossina palpalis palpalis. The pattern of activity, the isolation and partial characterization of this enzyme are described. Preliminary results on the interaction of trypsin activity and trypanosome development are presented and discussed.

The use of SIT in tsetse control campaigns implies mass release of sexually sterilized laboratory flies. For this purpose, large tsetse colonies are maintained using in vitro membrane feeding. Many studies concern the effect of gamma-irradiation on the vectorial capacity of the flies. Hitherto,

no studies have been undertaken to determine whether continuous in vitro rearing changes the susceptibility of flies to trypanosome infection. For this reason, we have compared G. palpalis palpalis from in vivo and in vitro colonies with respect to their susceptibility to infection with T. brucei brucei.

A. Interaction of trypsin activity and trypanosome development in the tsetse midgut

The role of digestive activity in the tsetse-trypanosome interrelation is still poorly understood. Gingrich et al. (1982, 1985) observed an increased midgut infection rate in flies given an infective feed with trypanosomes suspended in blood free from serum. He postulated a relationship between activity of midgut proteinases and the development of a procyclic infection.

A proteinase-parasite interaction was also proposed for other hematophagous insect vectors and their respective parasites. In Aedes aegypti trypsin-like proteinases are responsible for the destruction of ingested ookinetes of Plasmodium (Gass, 1977; Gass & Yeates, 1979; Yeates & Steiger, 1981). In Phlebotomus papatasi it was suggested that a specific component of the trypsin-like activity prevents the survival of Leishmania donovani and that modulation of this component by L. major enables it to thrive (Borovsky & Schlein, 1987).

The elimination of ingested trypanosomes in the midgut of G. palpalis palpalis was observed. Preliminary experiments were carried out to determine whether trypsin activity affects the establishment of a procyclic midgut infection. Trypsin activity was inhibited during the presence of an infective meal in the midgut. If trypsin affects the survival and/or transformation of bloodstream forms, the midgut infection rate would be changed by the inhibition.

Materials and Methods

Flies

Adult G. palpalis palpalis from the RUCA-stock colony were used in this study. The colony is maintained at the laboratory since 1974 and originated from wild pupae collected in Kaduna area (Nigeria). Flies were kept at 24.5 $\pm$ 1.0°C and 80 $\pm$ 5 % R.H.

Teneral flies were given an infective feed within 32 h. following emergence.

Trypanosomes

Trypanosoma brucei brucei (stock EATRO 1125) bloodstream forms were used throughout to infect flies.

Rate of trypanosome killing

Experimental flies were fed on an infested guinea-pig when the parasitaemia was at least $10^{7.8}$ trypanosomes/ml and were dissected at 24 h. intervals after infection. Midguts were examined for the presence or absence of trypanosomes using phase contrast microscopy (x400)

Inhibition-infection experiment

Using the method of Welburn & Maudlin (1987), infective feeds were given in vitro by suspension of frozen stabilates of the trypanosome in a 50/50 bovine/pig blood mixture at a concentration of 10^6-10^7 trypanosomes/ml.

Soybean Trypsin Inhibitor (1 mg/ml) was added to the infective meal. This dose was sufficient to inhibit all trypsin activity for more than 72 hours after feeding. Flies were maintained on non-infective blood prior to infection. Midguts were examined for trypanosomes 10 and 30 days post-infection.

Results and Discussion

Destruction of ingested trypanosomes occurred within 5-7 days after the infective feed (Figure 1). Thereafter, surviving trypanosomes were all procyclic forms.

The effect of trypsin activity inhibition on trypanosome development *in vivo* was evaluated (Figure 2).

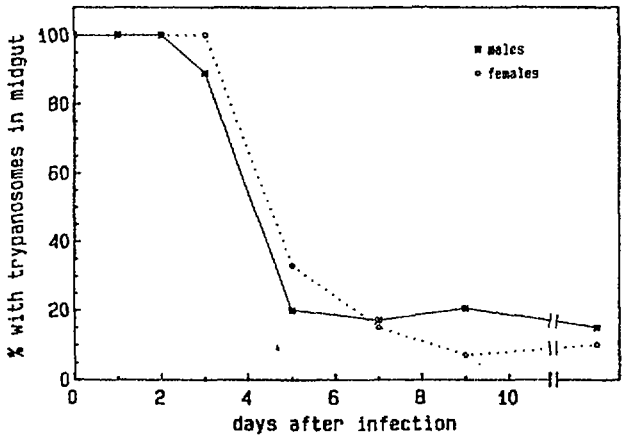

Figure 1. Elimination of ingested bloodstream forms of *T. brucei brucei* in the midgut of *G. palpalis palpalis*

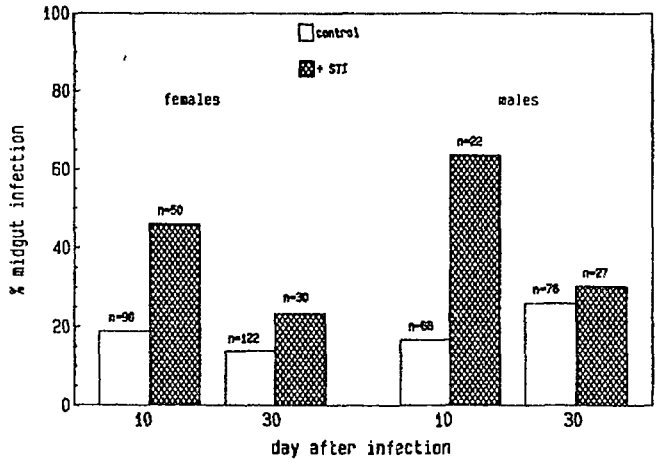

Figure 2. Midgut infection rate of flies given an infective feed with *T. brucei brucei* bloodstream forms together with Soybean Trypsin Inhibitor (1mg/ml). Midguts were examined for trypanosomes 10 and 30 days after infection

Ten days post-infection, trypanosomes occurred significantly more frequent in flies where trypsin activity was inhibited (X^2females:13.2, $p<0.001$; X^2males: 16.8,$p<0.001$). However, 30 days after the infective feed, no differences in midgut infection could be observed. The latter could indicate that the elimination and/or transformation of trypanosomes during the initial period after infection was retarded due to trypsin activity inhibition and was not yet finished on day 10 post infection.

Yabu & Takayanagi (1988) have demonstrated that transformation of bloodstream forms of a monomorphic strain of T. brucei gambiense is stimulated in vitro by trypsin treatment. Transformation of non-treated bloodstream forms and forms treated with soybean trypsin inhibitor were prolonged.

Lectin activity in the tsetse midgut was shown to be important in determining the susceptibility of the fly to trypanosome infection (Maudlin & Welburn, 1987; Welburn et al.,1989). Trypsin treatment removes VSG and other glycoproteins of the trypanosome surface. As a result lectin binding (Concanavalin A) on the membrane of bloodstream forms was enhanced (Balber & Frommel, 1988). Hence, trypsin activity in the tsetse midgut could possibly make the trypanosomes more vulnerable to the action of the midgut lectins.

Lectins, trypanolysins (Stiles et al.,in press), trypsin and possibly other unknown factors make the midgut environment hostile for an ingested trypanosome. However, the mode of action on the parasite and their role in determining the susceptibility to infection of a fly is still poorly understood. Valuable information could be obtained by evaluating the effects of these factors separately or combined on trypanosome development in an in vitro system. Therefore, purification of these molecules is needed.

B. Digestive activity in the tsetse midgut

Digestion of the bloodmeal takes place in the posterior region of the midgut. As proteins are the most important nutritive components of the meal, digestive activity is predominantly proteolytic. A comprehensive study on the proteolytic activity of the tsetse fly G. morsitans morsitans has already been carried out by Gooding (1976, 1977). Seven proteolytic enzymes have also been identified in the midgut of G. palpalis palpalis flies (Cheeseman and Gooding, 1985), namely trypsin and two trypsin-like enzymes, carboxypeptidase A&B and two aminopeptidases.

We have determined the quantitative contribution of a trypsin in proteolytic digestion. The activity pattern of the enzyme as response to feeding will be described. A two-step procedure for the isolation of tsetse trypsin has been developed. This has enabled us to isolate and characterize the enzyme.

Materials and Methods

Determination of activity pattern

Teneral and non-teneral flies, starved for 120 hours, were fed on a guinea-pig. Only fully engorged flies were retained for further measurements. Tsetse fly midguts were dissected in 0.15M NaCl prior to feeding as well as at increasing time (24, 48, 72, 96, 120 and 144 hrs.) following ingestion of a bloodmeal. For each sample, two midguts were pooled and stored at -20°C until analysis. Aliquots were thawed, homogenised in 200 ul 50 mM Tris/HCl buffer (pH 8.0, 4°C) and centrifuged at 12000g during 15 min. Supernatants were used for measuring enzyme activity.

Trypsin activity was measured by spectrophotometrical determination of the esterolytic action towards the synthetic substrate TAME (p-tosyl-L-arginine methylester). Molar extinction coefficient (δ_{247}=540) was used to determine the amount of substrate hydrolysed.

Selective inhibition

The contribution of trypsin in the proteolytic digestion was measured by means of selective inhibition. Prior to activity measurements, midgut homogenates were incubated during 30 min. with specific inhibitors. Soybean Trypsin Inhibitor (STI) 1mg/ml and 0.5mM TLCK (N-p-tosyl-L-lysine chloromethylketon) were used to inhibit respectively serine protease activity (trypsin and chymotrypsin) and trypsin activity. After incubation, remaining protease activity was determined by azocasein hydrolysis (sulfanilamide azocasein). Absorbance was measured at 366nm.

Protein determination

Protein concentration was measured by a modified method of Lowry (Peterson, 1977). Bovine Serum Albumin (Serva) was used as the protein standard.

Trypsin isolation

Posterior midguts were dissected 48 h. after a bloodmeal. Aliquots of 200 midguts were homogenised in 10 ml 50 mM Tris/HCl buffer, pH 8.0^{o}C and centrifuged at 100,000 g for 1 hour at 4^{o}C. Supernatant, designated as the crude midgut homogenate, was used in the isolation procedure.

After passage through a 0.2 um Millipore filter, the crude midgut homogenate was applied to a Sephadex G-75 column (2.6 x 80 cm). Elution was performed with a 50 mM Tris/HCl buffer (pH 8.0, 4^{o}C) at a flow rate of 55 ml/hr. Fractions with trypsin activity were pooled and concentrated by means of ultrafiltration on a Amicon PM-10 membrane.

This partial purified sample was further subjected to affinity chromatography. Soybean Trypsin Inhibitor (Sigma) was coupled to a CNBr-activated Sepharose 4B gel (Pharmacia) according to the manufacturer's recommendation. It was shown previously that the inhibitor used as ligand is very efficient in complexing all tsetse tryptic activity. Bound trypsin could be eluted with 0.1 M Glycine/HCl buffer (pH 2.5, 4^{o}C) containing 10 mM TAME. Under these acidic conditions, tsetse trypsin was still stable for several hours. The trypsin-active fractions were pooled and concentrated on a PM 10 membrane.

FPLC analysis

Purified sample was subjected to further analysis on the FPLC system (Pharmacia) using the Mono Q anion exchange column. Sample was applied to the column in 50 mM Tris/HCl buffer (pH 8.5, 25^{o}C). Elution was achieved at the same pH with a linear NaCl-gradient (0 - 0.5 M) at a flow rate of 1 ml/hr.

Electrophoresis

Polyacrylamide gel electrophoresis (PAGE) was conducted on 10 % gels. A discontinuous buffer system (electrode buffer: Tris/Glycine, pH 9.0 and gel buffer: Tris/HCl, pH 8.9) gave the best resolution.

SDS-PAGE was carried out on a 11% gel according to the method of Laemli (1970). Molecular weight standards were provided by Sigma.

Isoelectric focusing was performed on 1% agarose gels with a pH gradient generated of 3.5 to 10.0 (Ampholines - Pharmacia). Focusing conditions were as described by the manufacturer's manual. pI markers of Sigma were run simultaneously with the samples.

Proteins were stained with Coomassie Blue R 250. Specific trypsin activity was stained according to the method of Zwilling & Neurath (1981) but a tenfold lower concentration of the diazoniumsalt Fast Garnet GBC was used (0.4 mg/ml). Silver staining of the gels was performed by using the Sigma Silver staining kit.

Results and Discussion

Digestion is dominated by trypsin, contributing to more than 50% of the total proteolytic activity during digestion (Table 1).

Table 1. Effect of Soybean Trypsin Inhibitor (STI) and TLCK on total proteolytic activity in the posterior midgut of G. palpalis palpalis females: % inhibition is expressed against the activity present in homogenate treated with buffer alone

	% inhibition	
hours after feeding	+ STI	+TLCK
24	63±12	51±3
48	70±8	55±12
72	68±9	49±4

Trypsin activity follows a cyclic pattern after blood feeding (Figure 3). In teneral female flies, maximum activity is reached 48-72 hours after feeding and is then declining to the pre-ingestion level at about 120 hours. In non-teneral virgin females (14 days old) trypsin activity increases more rapidly and reaches a maximum 24 hours after feeding. Peak activity is considerably higher than in teneral flies (7.5 times versus 3.5 times the initial activity). A same tendency was observed for teneral and non-teneral males. Generally, male flies show a lower trypsin activity and slightly shorter digestive cycle (96-120 hours after feeding) than females (data not shown).

Differences in amount of trypsin at peak activity could be attributed to variations in meal size or amount of protein ingested. It was observed previously that male flies ingest smaller bloodmeals than females. Data on meal size of non-teneral flies were not collected.

Tenerals digest their bloodmeal more slowly than non-tenerals, possibly because in a freshly emerged fly the peritrophic membrane is not yet fully developed, and transport of the meal through the midgut is retarded. As a result trypsin will be released more slowly.

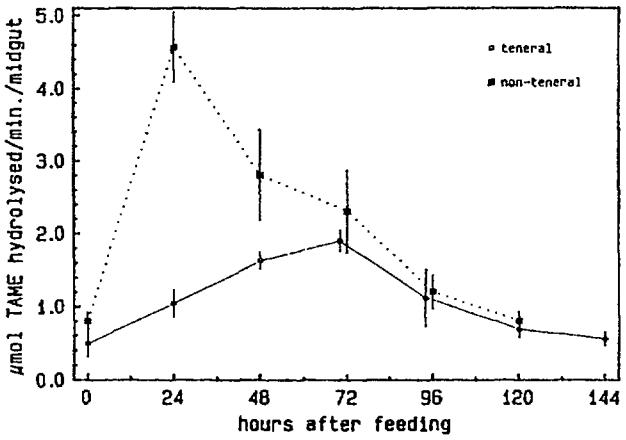

Figure 3. Trypsin activity in the posterior midgut of teneral and non-teneral G. palpalis palpalis females at various times after ingestion of a bloodmeal

Isolation of trypsin of the tsetse fly G. palpalis palpalis is achieved by a two step procedure (Table 2). Crude midgut homogenate is first subjected to gelfiltration on a Sephadex G-75 gel (Figure 4). Fractions with trypsin activity are eluted in a single peak (peak 3). The majority of bulk proteins are removed by this step (peaks 1 and 2). Pooled fractions containing trypsin activity are further purified by affinity chromatography on a Sepharose 4B gel with Soybean Trypsin Inhibitor as coupled ligand. The bound fraction could only be eluted by combining a low pH (Glycine/HCl buffer, pH 2.5) with an excess of the trypsin substrate TAME (10 mM).

Table 2. Isolation of midgut trypsin of G. palpalis palpalis females (600 midguts were used). 1 trypsin unit(U) is defined as the amount of enzyme required to hydrolyse 1/mol of TAME/min. at 30°C in a 50 mM Tris/HCl buffer pH 8.0

	Volume (ml)	Protein content (mg)	trypsin activity (U)	specific activity (U/mg)	yield (%)
1.Crude midgut homogenate	30.0	177.7	1190	7	100
2.Sephadex G-75 (+PM10)	25.0	11.8	1110	94	93
3.STI-Sepharose 4B (+PM10)	1.8	1.7	410	240	35

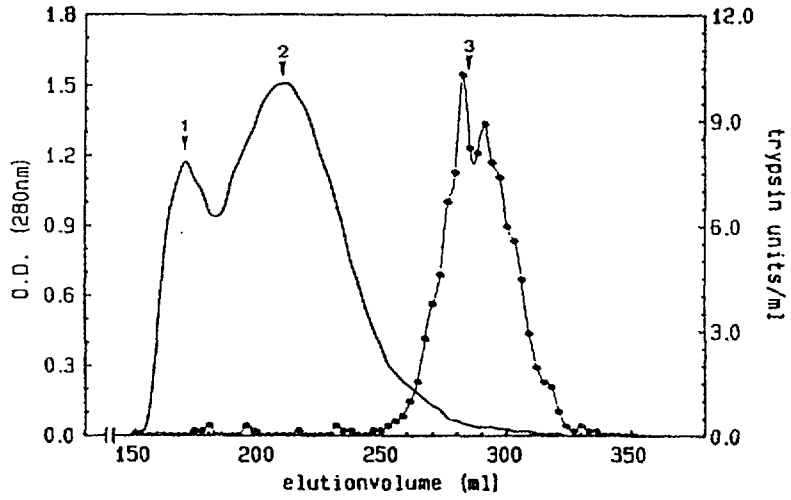

Figure 4. Gelfiltration on a Sephadex G-75 column of a crude midgut homogenate of G. palpalis palpalis females, 48 hours after feeding

Following this purification procedure about 35% of the initial trypsin activity is yielded. The specific activity is very high (240 units/mg) indicating a high grade of purification.

Chromatography of the obtained purified fraction on a Mono Q anion exchange column (FPLC system) reveals at least three distinct peaks with trypsin activity (Figure 5). However, major trypsin activity occur in the unbound fraction (peak 1). Separation must be improved to find out whether this fraction is homogeneous or consists of several proteins. Three major protein bands with trypsin activity were shown by native PAGE electrophoresis (Figure 6). A few non-trypsin contaminating proteins could only be detected by Silver staining (not shown). pI values of the proteins in the purified sample were estimated by isoelectric focusing. Three intense protein bands with trypsin activity could be detected with pI values of 8.6, 5.1 and 4.9. A minor protein band is observed with a pI value of 7.9. (Figure 7). SDS-PAGE reveals only two classes of different molecular weight, 24 and 26.5 kDalton (Figure 8), suggesting that the enzymes have very similar molecular weights.

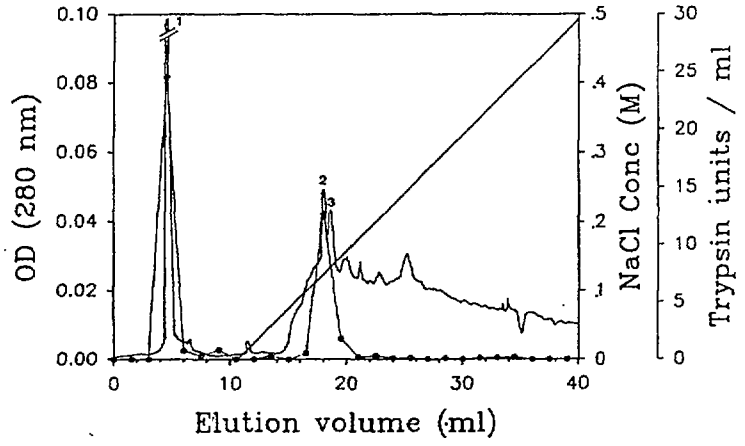

Figure 5 Separation of purified midgut trypsin of G. palpalis palpalis on a FPLC-Mono Q anion-exchange column

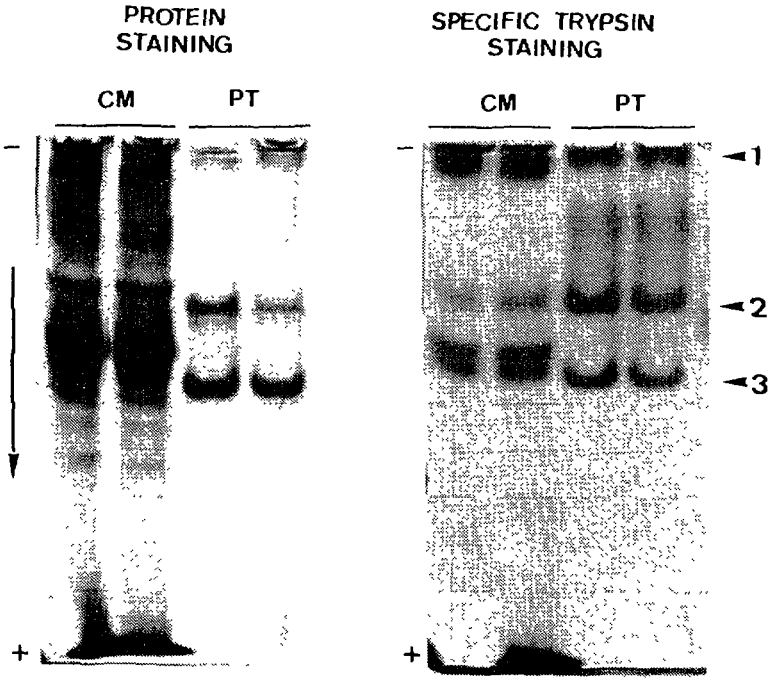

Figure 6. Native PAGE of purified midgut trypsin of G. palpalis palpalis

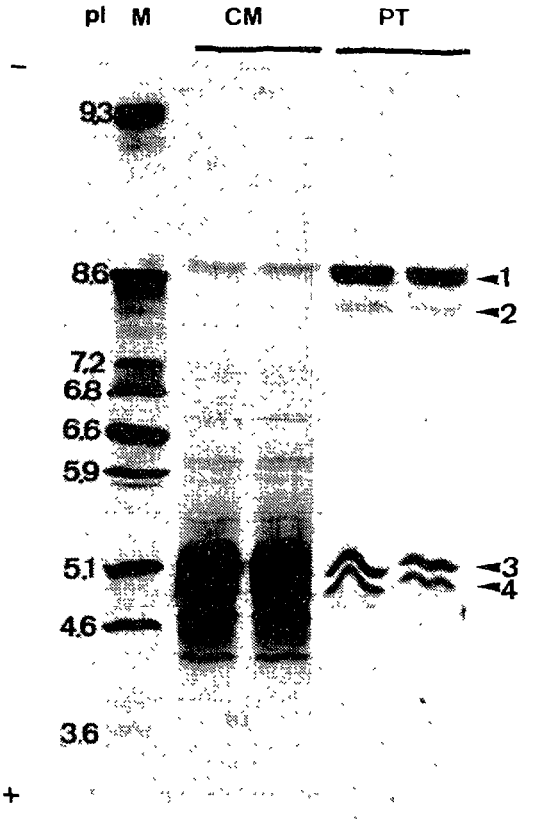

Figure 7. Iso-electric focusing of purified midgut trypsin of G. palpalis palpalis. (M=marker proteins; CM=crude midgut homogenate; PT=purified trypsin)

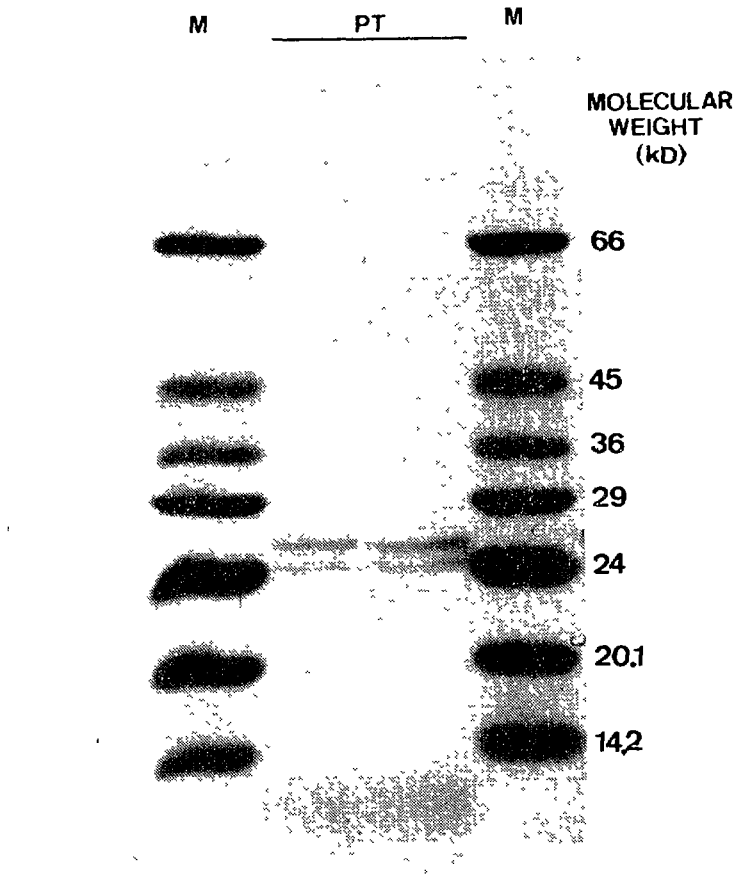

Figure 8. SDS-PAGE of purified midgut trypsin of G. palpalis palpalis. (M=marker proteins; PT=purified trypsin)

At least three different enzymes with trypsin activity are present in the posterior midgut of G. palpalis palpalis. Similarity of the trypsin banding pattern of the crude midgut homogenate and the purified sample indicates that this multiplicity is not an artefact generated by trypsin autodigestion or by manipulations during the isolation procedure.

These data confirm the results of Cheeseman & Gooding (1985) who identified a trypsin and two trypsin-like enzymes (proteinase VI and VIII) in G. palpalis adults by DEAE-cellulose chromatography and gelfiltration on a Sephadex G-100.

C. Susceptibility of in vivo and in vitro fed G. palpalis palpalis flies to infection with T. brucei brucei

Materials and Methods

Flies

Adult G. palpalis palpalis flies from the RUCA/Antwerp and from the IAEA/Vienna stock colonies were used in this study. Both colonies originated from the same wild pupae material, collected in Nigeria. The RUCA colony has been continuously maintained on guinea-pigs (since 1974) whereas the IAEA colony has been membrane-fed during the last 12 years. Flies were infected on a guinea-pig as described above. After the infective feed and until dissection, groups of flies were fed either in vivo on a guinea-pig or in vitro on bovine/porcine blood mixture (50:50).

Table 3. Infection rates of G. palpalis palpalis flies infected with T. brucei brucei (EATRO 1125). (M=% midgut infection; SG= % mature infection; (n)=number of flies dissected)

	FEEDING	RUCA G.p.p.	IAEA G.p.p.
FEMALES	in vivo	M: 13.7 (384) SG:3.6	15.4 (318) 0.03
	in vitro	25.2 (242) 0.0	30.6 (137) 0.0
MALES	in vivo	17.6 (306) 4.5	32.3 (232) 2.6
	in vitro	25.7 (206) 0.0	38.3 (115) 0.0

Results and Discussion

Infection rates are shown in table 3. Data were statistically analysed using a G-test. Midgut infection rates of females from both colonies were not significant different. However, male flies coming from the IAEA colony developed more easily a midgut infection. Differences in maturing of an infection were observed. In vitro-fed flies showed an apparent lower maturation rate of procyclic forms. However, we must take care for further interpretation because numbers were too small to do reliable statistics.

The type of diet affects significantly midgut and salivary gland infection rates. Establishment of a procyclic midgut infection is enhanced by feeding the flies on a bovine/porcine mixture through membrane. However, no mature infections were observed in these groups of flies.

Susceptibility of a fly to trypanosome infection is determined by variable factors (lectins, RLO's, lysins, trypsin, ...) appearing in the midgut. Maybe the type of diet or the way it is offered to the flies can make important changes on the long term on some of these factors. Preliminary analysis of trypsin activity revealed no differences between the in vivo and in vitro reared flies. The effect of the diet after the infective feed is remarkable. The results suggests that consecutive meal(s) can lead to changing conditions which interfere with trypanosome development in the midgut.

Conclusions

Various factors such as lectins, trypanolysins, digestive enzymes and possibly others make the midgut environment hostile for ingested trypanosome bloodstream forms. Trypanosomes have to conquer this battle and transform to adapted procyclic form.

Our own results of a preliminary in vivo study as well as data described in the literature, suggest that trypsin activity could be involved in the elimination and/or transformation of bloodstream forms. However, more convincing information should be obtained by determining the effect of isolated tsetse trypsin on trypanosome development in an in vitro system.

Hence, a method for purification of midgut trypsin of G. palpalis palpalis was developed. Characterisation of the enzyme revealed that at least three different enzymes with trypsin activity are present.

In vivo and in vitro reared G. palpalis palpalis were compared with respect to their susceptibility to infection with T. brucei brucei. In vitro reared flies seemed to establish more easily a procyclic midgut infection but had a lower rate of maturation of the infection. Further research on this phenomenon is needed.

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BIOSYNTHESIS OF LIPIDS IN FEMALE TSETSE FLIES* FOLLOWING MATINGS WITH RADIATION-STERILIZED OR NORMAL MALES

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Abstract

Tsetse females mated to radiation-sterilized males have a pattern of lipid synthesis identical to those mated normally through nearly all levels of lipid content except one. Synthesis of lipids from injected [^{14}C]-1,2-acetate shows no mating condition correlation for total lipids, total phospholipids, total neutral lipids or for major sub-classes of phospholipids or most neutral lipids. Only the 1,2-diacylglycerols show a distinct depression of synthesis and then only in the uterine gland of a female mated to an irradiated male. Thus without carrying lipid analyses to a level distinguishing isomeric forms in a specific type of tissue, one could not discern an ultimate difference in the lipid biochemistry of the abnormally mated female.

1. INTRODUCTION

Lipid metabolism of tsetse flies has held a measure of interest (perhaps beyond its importance) since the sterile insect technique (SIT) developed. Yet, the role of lipids in sterile females who mated with irradiated males is still unknown. Do those females, who mate with irradiated males, have a lipid synthesis pattern differing from those who mated with normal ones? And would such a difference be important? This question, albeit possibly not cogent in matters of tsetse control, is of fundamental importance in insect biochemistry. And these authors believe that fundamental science underlies the success of the SIT. Thus, the following experiments evolved.

A brief review of the literature about what we know of lipids in tsetse flies has revealed an interesting assortment of topics. But none focus on possible differences in lipid production by the unfortunate wild female who mates with one of our artificially-sterilized males. Does such a mating matter with regard to her biochemistry, vigor or longevity? Will her possible long-term vigor diminish the success of releasing radiation-sterilized flies to mate with wild females? And, too, can we use her pattern of lipid synthesis in some way to tell us in the field that she had had a successful, i.e., fertile, or an unsuccessful unfertile mating? This paper will attempt to give us some insights into these questions.

* *Glossina palpalis palpalis* (Robineau-Desvoidy) (DIPTERA:GLOSSINIDAE).

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2. MATERIALS AND METHODS

2.1. Tsetse handling techniques and protocols

The stock colony of *G. palpalis palpalis* maintained by the Entomology Unit at the FAO/IAEA Laboratory in Seibersdorf, Austria was the source of the flies used in this study. Rearing and handling conditions were essentially as described by Van Der Vloedt [1]. The flies were fed *in vitro* on freeze-dried blood through a silicone rubber membrane [2]. Males were selected at 10 days of age and given 12 Krad of gamma radiation from a cobalt-60 GammaCell. Four days later, they were placed in a 1 to 1 ratio with two-day-old virgin females and were allowed to mate for 5 to 7 days. An equal number of females were also mated with normal males under identical conditions.

Four experimental protocols of generally similar design were carried out. Females were injected and lipids obtained (described below) at 13, 15, and 19 days of age in replicates I, II and IV. In replicates II, III and IV, females were also injected, etc. at roughly 10-day intervals until no females remained (through ages 70, 80 and 82 for those three replicates, respectively).

2.2. Injection with radiolabeled precursors

Following mating, females were injected through the dorsal midline of the thorax to a depth of 5 mm at room temperature. For some preliminary experiments, each female received 0.25 μCi of [^{14}C]-U-leucine in 5.0 μL of sterile water. For all subsequent experiments, 0.5 μCi of [^{14}C]-1,2-acetate, sodium salt, was used. A metabolic holding period of 4 hours (only for preliminary experiments with both labeled leucine and acetate) or 24 hours followed.

2.3. Preparation of tissues

After the metabolic period, females were dissected in saline to obtain the following tissues for extraction of total lipids: fat body, uterine gland, larva *in utero* and the remaining carcass. Fat body and uterine gland were further processed to obtain essentially pure tissue by the methyl glyoxal procedure of Kabayo and Langley [3]. Preliminary experiments showed that this procedure did not affect the recovery of label or overall chromatographic pattern and identification of lipid sub-classes.

2.4. Lipid Analysis

2.4.1. Extraction of total lipids

Total lipids were extracted from the above four dissected tissue groups by the method of Bligh and Dyer [4] and brought to 0.3 mL in chloroform. Duplicate 10 μL of each total lipid extract were transferred to liquid scintillation vials, the chloroform was then removed by evaporation, and

radioactivity determined by liquid scintillation assay. The extracts were kept in chloroform under nitrogen in teflon-lined screw cap vials at -20^{0}C until analyzed.

2.4.2. Chromatography of lipids

Silica Gel 60 (Merck, Darmstadt) precoated thin-layer chromatography (TLC) plates were used throughout. The plates were first cleaned by dipping the lower edges into a tank containing anhydrous diethyl ether and allowing the fluid to advance to the top of the plate. Total lipids were analyzed for their neutral lipid content or their phospholipid content by employing one or the other of the following solvent systems. For neutral lipids, *n*-hexane:diethyl ether: glacial acetic and, 80:20:1, was used. In this system phospholipids remain at the origin and neutral lipid classes separate above. For phospholipids we used chloroform:anhydrous methanol:glacial acetic acid:saline (i.e., 0.9% NaCl in deionized water) 50:25:8:4. In this system neutral lipids travel to the top of the plate and phospholipid classes separate beneath.

For the analysis of diacylglycerols, we first separated the total lipid extract into gross neutral lipids and phospholipids on small silicic acid columns. A standard pasteur pipette plugged with glass wool at the bottom of the widest point was filled to a depth of 2 cm with silicic acid (Biosil HA, Biorad Corp., USA) slurried in chloroform. Each total lipid extract was applied to the top of a silicic acid column and the neutral lipid components were recovered in 3 washes of chloroform of about 1 mL each. A fresh silicic acid column was used for each total lipid extract to be separated. After suitable adjustment to known volumes, 10 µL aliquots were counted by liquid scintillation assay. The neutral lipids were then analyzed for their diacyl glycerol components by TLC using the following solvent system: *n*-hexane:diethyl ether:anhydrous methanol:glacial acetic acid, 80:20:20:1. In this system the 1,2- and 1,3- diacylglycerols clearly separate from one another and from all other neutral lipids including cholesterol.

2.4.3. Radioassay of separated lipid components

Lipid components were made visible in iodine vapor and their positions were marked on the TLC plates. Their identities were confirmed by comparison with the positions of known authentic standards spotted in adjacent lanes. After evaporation of the iodine, each area of silica gel corresponding to a known lipid class was carefully scraped off the plate and transferred to a liquid scintillation vial. Ten mL of a toluene-based solution containing BBOT (after Snyder [5]) were added to each vial and radioactivity determined by liquid scintillation.

2.4.4. Liquid scintillation counting

The Beckman LS 7800 liquid scintillation spectrometer was used throughout. It was calibrated for automatic external standard quench correction using [^{14}C]-toluene in the BBOT liquid scintillation solution containing varying amounts of neutral and phospholipids or acetone as quenching agents.

2.5. Chemicals and Standards

All chemicals were reagent grade or better. Authentic lipid standards were of the highest purity obtainable and were purchased from Applied Science Laboratories, USA; Pierce Chemical Co., USA; Serva, Heidelberg, W. Germany; Sigma Chemical Co., USA; and Supelco Inc., USA. The radiolabeled precursors came from Amersham International, UK, and New England Nuclear, USA.

3. RESULTS AND DISCUSSION

3.1. Choice of precursor

Incorporation of carbon-14 label into lipids from labeled leucine or acetate at various post-injection times is shown in Table I. Clearly acetate gives noticeably higher incorporation in 4 hours than leucine. Furthermore, a 24-hour incubation time gives better labeling than 4 hours, especially so in fat body and larvae. Thus, for all subsequent experiments, labeled acetate and 24 hours were the conditions used. McCabe and Bursell [6] showed that amino acids of all categories provided material for triacylglycerol synthesis via their contribution to the acetyl CoA pool in the male fly. We presume no differences in precursor preference would exist for the female fly. Their work strongly suggests, and our results here with female flies demonstrate that labeled acetate is clearly a preferred precursor over amino acids, which curiously had been the precursors chosen for much of the earlier work on tsetse lipid biosynthesis. This choice was likely predicated by the fact that the blood meal contains such a high protein, and upon digestion, amino acid content that amino acids would seem to be better precursors to study the nutritional assimilation of the adult's food. Yet when one wishes to investigate lipid biosynthesis, *per se,* it is clear that acetate, the most common lipid precursor, should be chosen. It would also be quite acceptable to use a labeled lipid, e.g., [^{14}C]-1-fatty acid in examining the synthesis of specific lipid subclasses containing acyl groups but such was not the sole object of the present work. Such fatty acid precursors, for example palmitic acid, have been used in several studies of lipid synthesis in the tsetse fly (see, e.g., Langley and Bursell [7]).

3.2. Distribution of label to total lipids of various tissues

To assess if there were differences in incorporation of label from acetate into lipids, a series of analyses were made beginning with the total lipid fraction. A condensed synopsis of this total lipid assessment is presented in Table II. Radioactivity (expressed as dpm corrected for tissue source difference) throughout the life span shows a steady decline as the flies age but shows no significant differences whether the flies mated normally or with irradiated males. Clearly fat body is the principal site of lipid synthesis and generally shows much higher rates of label incorporation when compared to uterine gland. Also it is clear that a considerable synthesis of lipids takes place in the

TABLE I. INCORPORATION OF CARBON-14 (dpm) INTO TOTAL LIPIDS FROM LABELED LEUCINE VS. ACETATE (15-day-old ♀♀) AT 4 AND 24 HOURS POST-INJECTION

TOTAL LIPID SOURCE	dpm* [^{14}C]-U-LEUCINE	dpm* [^{14}C]-1,2-ACETATE
	4 HOURS	
Fat Body	166	1712
Uterine Gland	634	3683
Larva	40	226
	24 HOURS	
Fat Body	not done	5532
Uterine Gland	" "	4373
Larva	" "	921

*per 10 µL volume adjusted total lipid.

TABLE II. INCORPORATION OF LABEL FROM ACETATE INTO TOTAL LIPIDS OF VARIOUS TISSUES AS A FUNCTION OF FEMALES' AGE WHETHER MATED NORMALLY OR TO IRRADIATED MALES

AGE (days)	MATING**	dpm* FAT BODY	UTERINE GLAND	LARVA	REMAINDER
10	I	31,600	2,700	-	22,700
	N	42,000	3,500	-	30,600
20	I	12,600	3,400	-	9,300
	N	7,200	4,100	10,900	9,400
40	I	4,400	2,100	-	8,100
	N	4,300	2,300	13,184	9,200
60	I	3,900	1,700	-	8,900
	N	1,600	3,600	11,900	11,000
80	I	7,800	3,400	-	6,300
	N	1,700	1,700	7,936	7,200

*dpm rounded to nearest 100.

**I: mated to irradiated male; N: to normal male

remainder of the body in many instances exceeding that occurring in the fat body. About the only discernable difference between females mated to normal males versus irradiated ones, is that when a larva is present, less radioactivity is present in the fat body. Presumably lipids synthesized at that site had been transferred to, and modified in, the uterine gland and were passed on to the larva as it fed on the uterine gland secretion. It is interesting to realize that females who will never a carry a larva *in utero,* still retain a lipid synthesis and metabolic system throughout life comparable to normal ones, at least at this general level of lipid synthesis and composition.

3.3. Comparison of synthesis of phospholipids and neutral lipids

Total lipids were next separated into phospholipids (PL) and neutral lipids (NL). Table III is a summary of a portion of our findings for 10- to 20-day intervals. (We also have data for intervals between those in this table, but since they do not differ materially from those shown, they have been omitted for clarity and simplicity.)

Clearly the fat body is a principal site of neutral lipid synthesis. Although the total dpm declines with age (as seen in Table II), the percent distribution of carbon-14 label to NL and PL is essentially constant. Lipid radioactivity in the uterine gland, on the other hand, differs significantly from that seen in fat body. Here, the dpm in the NL drops sharply making the percentage (but not the dpm) in the PL appear to rise. These patterns of incorporation and percent distribution of radioisotope do not appear to be correlated with mating status, i.e., those females who mated with irradiated males retain a normal pattern of PL and NL synthesis throughout their life even though they will never be capable of producing a larva.

These findings are in agreement with earlier work in other laboratories. For example Langley and Pimley [7] reported that the fat body synthesizes lipids in early pregnancy, but before the larva hatches, those lipids undergo a massive transfer to the uterine gland. There, they are incorporated into the secretion of this gland, the so-called larval "milk", for nourishment of the larva in the uterus. Later, Langley and Bursell [8] showed that the lipids synthesized in the fat body consisted mainly of triacylglycerols (formerly called triglycerides).

In the larva about 10 times more isotope is showing up in NL as opposed to PL. It is probable that the NL are direct larval dietary products produced by the secretion of the uterine gland. The PL are more likely due to *de novo* synthesis of cellular constituents by the growing larva utilizing radiolabeled NL from the uterine gland nutriment.

Interestingly, the remainder of the female fly also shows considerable synthesis of NL and in some cases PL. Before interpretations of lipid synthesis in all the tissues and especially in the remainder could be made, additional analyses were needed. These are summarized in the subsequent sections.

TABLE III. DISTRIBUTION OF LABEL TO PHOSPHOLIPID VS. NEUTRAL LIPID SUBCLASSES AS A FUNCTION OF AGE AND MATING STATUS

AGE (DAYS)	IRRADIATED MATING PL* dpm**	%	NL dpm	%	NORMAL MATING PL dpm	%	NL dpm	%
				FAT BODY				
10	560	4.2	12,940	95.8	720	3.5	19,750	96.5
20	270	3.5	7,360	96.5	240	6.4	3,570	93.4
40	310	7.9	3,600	92.1	210	8.0	2,390	92.0
60	393	14.4	2,340	85.6	140	10.4	1,190	89.6
80	360	6.1	5,520	93.9	150	17.3	700	82.6
				UTERINE GLAND				
10	610	39.1	950	60.9	560	23.1	1,850	76.9
20	720	30.8	1,630	69.2	520	20.6	2,000	79.4
40	850	39.4	1,310	60.6	440	30.2	840	69.8
60	650	43.1	860	56.9	590	21.3	2,180	78.7
80	680	31.7	1,460	68.3	160	26.2	450	73.8
				LARVA				
20	--	--	--	--	440	8.2	4,960	91.8
40	--	--	--	--	380	8.2	4,270	91.8
60	--	--	--	--	520	11.6	3,920	88.4
80	--	--	--	--	340	11.2	2,700	88.8
				REMAINDER				
10	3,080	31.0	6,850	69.0	2,690	20.2	10,550	79.7
20	1,060	17.1	5,130	82.9	860	20.9	3,260	79.1
40	930	20.0	3,730	80.0	600	19.3	2,530	80.7
60	850	20.1	3,390	79.9	1,220	31.4	3,100	68.6
80	1,200	21.7	4,350	78.3	560	39.2	870	60.8

*PL: phospholipids; NL: neutral lipids.

**dpm rounded to nearest 10.

3.4. Principal component sub-classes of phospholipids and neutral lipids in tissues of tsetse females

Using the TLC systems described in Materials and Methods, and by comparison with the Rf's and positions of authentic standards, we identified the major and minor lipid sub-classes as listed in Table IV.

From this list it is clear that the spectrum of lipid classes present in these tissues does not differ from that seen in most other insects. The major classes in the table also were those incorporating radioisotope from the labeled precursors. Some components (e.g., cholesterol) were easily detected by exposure to the iodine vapor, but contained no apparent radioactivity of their own.

TABLE IV. PRINCIPAL COMPONENTS OF PHOSPHOLIPIDS AND NEUTRAL LIPIDS OF SELECTED TISSUES OF THE FEMALE TSETSE FLY

PHOSPHOLIPIDS		NEUTRAL LIPIDS	
MAJOR*	MINOR	MAJOR*	MINOR
phosphatidyl cholines (PC)	phosphatidyl serines	diacylglycerols (DAG)	unsterified fatty acids
phosphatidyl ethanolamines (PE)	phosphatidyl inositols	triacylglycerols (TAG)	monocylglycerols
	phosphatidic acids	hydrocarbons (HYD)	cholesterol
	lyso phosphatidyl cholines		steryl esters
	lyso phosphatidyl ethanolamines		other unidentified
	sphingomyelins		

*Abbreviations of major components in parentheses; these are also used in the text.

Two phospholipids, namely phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE), were the dominant phospholipids. Diacylglycerols (DAG) and triacylglycerols (TAG) were the principal neutral lipids in fat body, uterine gland and the larva. However a third NL class, hydrocarbons, was identified in the remainder of the carcass and, as later shown, incorporated a significant amount of carbon-14 from the labeled precursor, acetate. Hydrocarbons have been reported as a constituent of *G. morsitans,* in lipid extracts of mixed sexes [9], and are generally known to be synthesized in insects from injected labeled precursors.

3.5 Synthesis patterns of the major phospholipids and neutral lipids

Patterns of incorporation of isotope into the major PL and NL lipid sub-classes are summarized in Table V. We also have data from intervening time intervals which do not differ significantly from those intervals presented here. As before, we chose to omit them for clarity and simplicity.

The fat body incorporates radioactivity principally into PC and PE, with the former in excess by about 2:1 over the latter. In the NL, the major component synthesized is TAG. Recalling that NL synthesis predominates over PL synthesis in this tissue, it is clear that the major role of the fat body is to synthesize the energy rich storage lipid class, the TAG's. No strong differences appear to exist between normally mated females versus those mating with irradiated males.

The incorporation pattern is similar in the uterine gland for the PC, but abruptly different in the NL sub-classes where a sharp rise in labeling of the DAG's is clearly noted. Thus this gland is apparently receiving labeled lipids from the fat body via the haemolymph as a DAG bound as a lipo protein. It perhaps also synthesizes DAG and TAG *de novo.* This gland therefore reflects a modified spectrum of lipids in undergoing its role of preparing nutrients for the larva in the uterus.

Once again, no obvious correlations with mating status appear to exist, i.e., females without a larva or the possibility of ever having one, have the modified lipid content of their milk just as do the normally-mated females.

The labeling pattern in the larva is yet again different from the principal patterns of maternal lipids. Here the two phospholipid classes have nearly equal amounts of labeling, probably signifying a very active synthesis of cellular components during larval growth and development. The incorporation of label into the DAG's is even higher than that noted for the uterine gland. About 40% of the label is in this fraction, the balance being in the TAG's. D'Costa and Rutesasira [10] reported that larva of *G. morsitans* near pupation contain 60 percent triglycerides and 38 percent diglycerides, figures which are consistent with the values given here in Table V.

The rest of the female body also synthesized lipids. The pattern of incorporation into the PL's is roughly the same as for fat body and uterine gland with PC predominating over PE in nearly all instances. Synthesis of NL, however, is quite different because of the incorporation of large amounts of label (up to nearly 50%) in the hydrocarbon fraction. Such synthesis of these components is well-documented in insect physiology and biochemistry (see Ref. [1]) but is of little apparent significance in the present comparison of the mating status of the females.

3.6. Synthesis of diacylglycerol isomers by the various tissues

Because of the striking increase in DAG's in uterine gland and larvae over that noted in the fat body or the remainder of the female's body, we decided to do a more detailed chromatographic analysis of those two tissue lipid extracts. This was carried out only on Replicate II. The results are shown in Table VI for the uterine gland and larva.

In all instances where complete separation of the two isomers was achieved, the 1,2 isomer dominated over the 1,3 form. Langley et al. [11] showed that a portion of haemolymph lipid of *G. morsitans* was 1,2-diacylglycerol, and that with good separation of the two isomeric forms, very little radioactivity was found in the 1,3 isomer in flies given either labeled leucine or palmitate. Our results here with acetate-injected females reflect the dichotomy in the preference for synthesis of the 1,2-isomer. The role of diacylglycerols in lipid transport in haemolymph has been well documented [12], and the DAG's are usually complexed with a haemolymph lipoprotein (see Ref. [11]). Furthermore, it is well established that lipids acylated in the 1 and 2 positions, particularly DAG's and phosphatidic acids, are key intermediates in the synthesis and breakdown of TAG's, and similarly in the synthesis of phosphoglycerides containing choline, ethanolamine, serine or inositol. Diacyl glycerols with the the 1,3 configuration do not participate in such key metabolic pathways as do those with the 1,2 form [13].

TABLE V. PERCENT DISTRIBUTION OF THE MAJOR PHOSPHOLIPID AND NEUTRAL LIPID CLASSES OF VARIOUS TSETSE FEMALE TISSUES AS A FUNCTION OF AGE AND MATING STATUS

AGE (days)	MATING	dpm (%) PHOSPHOLIPIDS PC*	PE	NEUTRAL LIPIDS DAG	TAG	HYD
		FAT BODY				
10	I	54.4	25.4	2.1	94.9	
	N	52.3	15.5	1.8	97.0	
20	I	72.3	28.2	3.6	89.0	
	N	50.3	25.2	4.2	85.0	
40	I	46.5	29.6	4.9	76.3	
	N	42.2	29.8	6.0	84.0	
60	I	44.6	28.7	6.2	70.7	
	N	42.2	32.5	7.8	83.9	
80	I	63.2	23.9	3.2	81.1	
	N	42.2	29.2	7.2	86.7	
		UTERINE GLAND				
10	I	48.3	17.6	13.5	86.5	
	N	5.8(?)	18.0	7.8	83.6	
20	I	49.6	28.3	16.6	78.2	
	N	51.6	21.0	27.0	78.2	
40	I	36.6	27.8	15.1	73.8	
	N	44.1	23.0	30.8	61.6	
60	I	45.6	12.4	19.6	67.2	
	N	56.7	21.7	10.5	83.8	
80	I	64.2	26.3	12.4	83.8	
	N	73.6	29.4	23.9	76.1	
		LARVA				
20	N	34.6	31.0	42.8	52.8	
40	N	32.0	33.6	44.1	53.6	
60	N	52.0	35.2	27.9	68.5	
80	N	36.0	32.4	34.7	61.8	
		REMAINDER				
10	I	36.3	12.6	3.9	55.6	27.3
	N	53.0	14.5	3.1	76.2	16.3
20	I	45.4	25.2	4.5	42.7	41.0
	N	32.9	18.5	8.1	51.1	39.5
40	I	37.1	14.1	5.2	35.9	40.6
	N	42.0	14.2	8.3	35.0	48.6
60	I	41.0	20.0	5.8	32.7	41.4
	N	31.9	10.0	11.7	26.9	45.0
80	I	39.8	11.0	6.0	40.7	35.5
	N	36.1	21.0	22.4	56.9	(?)

*Abbreviations are identified in TABLE IV.

TABLE VI. SYNTHESIS OF 1,2- VERSUS 1,3-DIACYLGLYCEROLS IN UTERINE GLAND AS A FUNCTION OF AGE AND MATING STATUS AND LARVAE AS A FUNCTION OF AGE. FIGURES ARE CARBON-14 dpm % INCORPORATED

AGE (days)	IRRADIATED MATING 1,2- DAG	1,3- DAG	NORMAL MATING 1,2- DAG	1,3- DAG
		UTERINE GLAND		
13	5.3	1.3	6.1	1.1
15	8.8	2.5	12.1	0.8
19	7.0	2.7	23.7	2.6
29	10.8	5.0	24.5	2.2
40	10.2	2.5	25.4	3.6
50	10.1	3.8	21.0	11.0
61	6.9	3.8	23.0	1.8
70	9.5	4.1	NO INSECTS LEFT	
		LARVA		
15			26.3	2.1
19			43.1	1.4
29			35.7*	
40			45.9	
50			42.6	
61			40.0	

*Separation of the two isomers not achieved.

In the uterine gland of irradiated matings, the ratio of the 1,2 isomer over the 1,3 was as much as 4 to 1. However in this gland from normally-mated females the 1,2 isomer dominated by as much as 11 to 1 over the 1,3 isomer. In larvae of 19 days of age, the disparity between the two isomeric forms were even more striking, approximately 40 to 1. The differences in ratio of dominance of the 1,2 isomer between uterine gland DAG's of normal versus abnormally-mated females is the only correlation found in this research program associated with mating status. Lipids at all other levels analyzed appear to be synthesized by the female tissues equally without regard to mating condition. This finding therefore opens up the realization that it may be necessary to separate and analyze various molecular species of PC, PE, DAG, TAG and possibly other lipid classes before meaningful differences in the lipid biochemistry of abnormally mated flies can be found, and that such analyses need to be done on the lipids of specific tissues and at specific times in the ovarian cycle, and following known feeding intervals.

4. CONCLUSIONS

The overall pattern of lipid synthesis was examined in females of *Glossina palpalis palpalis* mated with normal males of radiation-sterilized ones. Starting with total lipids and progressing through several sub-classes of phospholipids and neutral lipids, we could find, with one exception, no essential differences throughout the life of the female mated to irradiated males versus normal ones in their capacity to synthesize the spectrum of lipids needed in the reproductive cycle. The single exception occurred in the diacylglycerols. Synthesis of the 1,2- DAG fraction was noticeably suppressed in the uterine gland in females having abnormal matings compared to females who bore a larva in the uterus. It was necessary, therefore, to analyze lipids to a level just above that of molecular lipid species in a specific tissue to find an alteration in lipid synthesis that correlated with mating condition. Were one to look only at total lipids, total phospholipids, phosphatidyl cholines, phosphatidyl ethanolamines or triacylglycerols, or lipids from whole flies, the ultimate effect of the abnormal mating would likely go undetected.

In most respects, therefore, the abnormally mated female maintains a lipid synthesis system or a parity with the normal female. From her own metabolic standpoint, she does not lose the ability to synthesize the lipid constituents necessary to support her life. Judged solely by her lipid metabolism, one could conclude that she is likely to be just as vigorous as her normal counterpart. Though she will never contribute to future tsetse populations, she remains in the wild, biting, feeding, transmitting disease organisms, and probably being a contender for her ecological niche as if she were normal. Had she not only to have been a non-producer, but also to have dropped out of the population at a younger age because of broadly altered lipid metabolism could have possible consequences on the success of controlling this fly by the SIT.

The question has been posed by others (in personal communication) whether we could somehow tell whether or not a female in the wild had had a successful mating. The answer appears to be yes, but only if analytic techniques capable of separating and quantitating the amounts of 1,2-diacylglycerols in the uterine gland are employed. Furthermore, the work reported here and by others shows that the timing of that analysis would have to correlate with the stages in each ovarian cycle and the feeding interval be known to assure a meaningful diagnosis of her mating condition. Just how practical and useful such a complex procedure might be under field conditions surely is open to considerable uncertainty.

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COMPARATIVE STUDY ON THE SUSCEPTIBILITY OF DIFFERENT *Glossina* SPECIES TO *Trypanosoma vivax, T. congolense* OR *T. b. brucei* *(Summary)*

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The present study was undertaken to determine if there were differences in the innate susceptibility of seven different laboratory-bred Glossina species and subspecies belonging to the three main taxonomic groups, morsitans, palpalis and fusca, originating from different parts of Africa, to Trypanosoma vivax isolated in Kenya and Nigeria. to T. congolense isolated in Tanzania and Nigeria, and to T. b. brucei isolated in Tanzania and Nigeria.

The morsitans group of tsetse used were G. m. centralis originating from mainland Tanzania and G. austeni from Zanzibar; the palpalis group were G. p. palpalis from Nigeria, G. p. gambiensis from Burkina Faso, G. f. fuscipes from Central African Republic and G. tachinoides from Chad; the fusca group tsetse was G. brevipalpis from Kenya.

T. vivax IL 2241 used was isolated from a cow in Likoni, Kenya; T. vivax 2337 from a steer on a cattle ranch in Galana, Kenya; T. vivax IL 3026 from an ox in Bamburi, Kenya; T. vivax Zaria Y486 from a Zebu cow in Nigeria and T. vivax EATRO 1721 was isolated from G. m. submorsitans in Nigeria. T. congolense IL 2047 used was isolated from a lion in Serengeti, Tanzania and T. congolense IL 2281 from a cow in Nigeria. T. b. brucei IL 923 was isolated from a Coke's hartebeest in Serengeti, Tanzania and T. b. brucei IL 2380 from a naturally infected pig in Nigeria. Both these stocks were found to be sensitive to the human serum.

Teneral tsetse from the seven production colonies were allowed to feed simultaneously for 24 days on Boran cattle infected with T. vivax, for 24 days on goats infected with T. congolense, or for 34 days on goats infected with T. b. brucei from east or west Africa. The tsetse were then dissected. Infection rates were as follows: 0-97.1% for T. vivax; 0.3-49.2% for T. congolense and 0-40.4% for T. b. brucei. Table 1 shows that cyclical development of both the Likoni and the Galana T. vivax was best in G. m. centralis (61.1%, 32.2%) and in G. brevipalpis (75.3%, 58.2%) but poor in G. austeni (1.8%, 5.0%) and the four palpalis group tsetse (range, 0-4.9%). For the Bamburi T. vivax the infection rates were high in all seven tsetse used (range, 16.3-91.3%). For the Nigerian T. vivax (Table 2), the infection rates were also high in all the seven tsetse examined, which showed a range of 55.5% to 97.1%. Table 3 shows that cyclical development of both the Tanzanian and the Nigerian T. congolense was best in G. m. centralis (35.3%, 49,2%), poorest in G. austeni (2.0%, 3.0%) and the four palpalis group tsetse (range, 0.3-6.0%), with G. brevipalpis intermediate (15.7%, 6.3%). Table 4 shows that infection rates of both the Tanzanian and the Nigerian T. b. brucei were high in G. m. centralis (40.4%, 6.8%), but low in the other tsetse species (range, 0-2.1%). Thus, there were differences in the innate susceptibility of different Glossina species and subspecies to T. vivax, T. congolense and T. b. brucei populations used, and futhermore, some trypanosome stocks developed better than others in the tsetse vectors.

In conclusion, cyclical development to metacyclics of T. vivax, T. congolense or T. b. brucei in tsetse involves a complex interactions amongst certain biological properties of the trypanosome population taken with a host's infected bloodmeal by a tsetse fly and some aspects of the physiology of the vector.

TABLE 1

Infection rates in different *Glossina* species and subspecies for *Trypanosoma vivax* isolated in Kenya

Tsetse species	Origin of *T vivax*	Number tsetse dissected	Infection rates (%)	
			Labrum	Hypopharynx
G m centralis	Likoni	379	66.9	61.1
	Galana	285	44.4	36.2
	Bamburi	80	91.3	91.3
G austeni	Likoni	374	2.5	1.8
	Galana	261	7.1	5.0
	Bamburi	100	69.0	69.0
G p palpalis	Likoni	344	0.0	0.0
	Galana	165	1.0	0.5
	Bamburi	80	16.3	16.3
G p gambiensis	Likoni	416	3.4	1.3
	Galana	199	8.9	4.9
	Bamburi	91	50.5	48.4
G f fuscipes	Likoni	332	0.8	0.5
	Galana	95	0.8	0.0
	Bamburi	66	31.8	28.8
G tachinoides	Likoni	389	0.5	0.3
	Galana	199	1.6	0.4
	Bamburi	67	16.4	16.4
G brevipalpis	Likoni	391	81.1	75.3
	Galana	228	67.6	58.2
	Bamburi	88	86.4	85.2

TABLE 2

Infection rates in different *Glossina* species and subspecies for *Trypanosoma vivax* isolated in Nigeria

Tsetse species	*T vivax* stabilate used	Number tsetse dissected	Infection rates (%)	
			Labrum	Hypopharynx
G m centralis	Zaria Y486	262	88.6	72.5
	EATRO 1721	240	96.4	92.6
G austeni	Zaria Y486	242	65.4	55.5
	EATRO 1721	185	80.0	71.4
G p palpalis	Zaria Y486	210	93.1	89.5
	EATRO 1721	157	83.1	78.6
G p gambiensis	Zaria Y486	247	94.0	91.9
	EATRO 1721	200	94.3	91.6
G f fuscipes	Zaria Y486	225	90.0	82.1
	EATRO 1721	128	96.0	87.2
G tachinoides	Zaria Y486	248	96.0	91.2
	EATRO 1721	173	94.3	93.7
G brevipalpis	Zaria Y486	280	93.3	81.7
	EATRO 1721	155	97.7	97.1

TABLE 3

Infection rates in different *Glossina* species and subspecies for *Trypanosoma congolense* isolated in Tanzania and Nigeria

Tsetse species	Origin of *T congolense*	Number tsetse dissected	Mean infection rates (%)		
			Midgut	Labrum	Hypopharynx
G m centralis	Tanzania	374	49.3	36.5	35.3
	Nigeria	421	63.5	49.9	49.2
G austeni	Tanzania	377	14.4	3.4	2.0
	Nigeria	419	14.6	3.4	3.0
G p palpalis	Tanzania	340	10.2	0.8	0.5
	Nigeria	286	18.7	0.7	0.7
G p gambiensis	Tanzania	339	5.9	0.4	0.3
	Nigeria	363	15.6	2.2	0.3
G f fuscipes	Tanzania	273	13.2	6.7	6.0
	Nigeria	301	9.2	3.2	0.9
G tachinoides	Tanzania	357	7.2	0.2	0.3
	Nigeria	401	9.8	2.5	2.0
G brevipalpis	Tanzania	354	33.8	17.6	15.7
	Nigeria	331	40.3	15.4	6.3

TABLE 4

Infection rates in different *Glossina* species and subspecies for *Trypanosoma brucei* isolated in Tanzania and Nigeria

Tsetse species	Origin of *T b brucei*	Number tsetse dissected	Mean infection rates (%)	
			Midgut	Salivary glands
G m centralis	Tanzania	357	80.7	40.4
	Nigeria	440	36.4	6.8
G austeni	Tanzania	426	5.4	0.2
	Nigeria	419	0.9	0.0
G p palpalis	Tanzania	353	3.7	0.4
	Nigeria	353	1.5	0.0
G p gambiensis	Tanzania	436	2.3	0.4
	Nigeria	391	0.4	0.0
G f fuscipes	Tanzania	302	5.6	2.1
	Nigeria	279	0.8	0.0
G tachinoides	Tanzania	355	3.3	1.0
	Nigeria	372	2.2	0.7
G brevipalpis	Tanzania	402	59.3	0.2
	Nigeria	402	18.1	0.0

VECTORIAL CAPACITY OF *Glossina palpalis gambiensis* AFTER COMBINED RADIATION AND LOW TEMPERATURE TREATMENT DURING THE LATE PUPAL STAGE

(Summary)

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Glossina palpalis gambiensis is one of the four tsetse fly species (together with G. tachinoides, G. m. submorsitans and G. longipalpis) occuring in Mali, where, as is the case in other countries of West Africa, it is an important vector of trypanosomiasis. The species is being maintained for a long time at the CRTA rearing centre, and is one of the three species for which the SIT was successfully used in the Sidéradougou eradication campaign. Plans are in preparation to use flies from CRTA to mount an integrated control campaign with SIT component in the Tienfala-Baguineda agro-pastoral zone of Mali. Therefore, after preparatory work at the FAO/IAEA Seibersdorf Laboratory, series of fly infectivity studies were conducted at CRTA, using materials from the local stock colony and available trypanosome stabilates: Trypanosoma vivax (Zaria 81/486/699 and Banan 85/CRTA/78), T. congolense (Karankasso 83/CRAT/57) and T. b. brucei (Farakoba 80/CRTA/1).

Late stage G. p. gambiensis pupae (25 or 28 or 30 days following larviposition) were exposed to a combined regimen of gamma radiation (Caesium 137; dose rate 500 Gy/hr; treatment in air with either 60 or 80 or 100 Gy) and low temperature (i.e. 15°C for 5 days either before or immediately after the radiation treatment).

Records were kept of the emergence rate, duration of pupal period, and sex ratio according to the various experimental regimen (Table 1). Random samples (100-120 flies per test series) of teneral or non-teneral male flies were allowed to feed on infected animals (goat infected with T. vivax or rabbits infected with T. congolense or T. b. brucei) at peak parasitaemia (T. vivax: 80 trypanosomes/field; T. b. brucei: 12 trypanosomes/field). Non-tenerals took clean bloodmeals (day 2 and 3 following emergence) before infected bloodmeals were offered on day 4, 5 and 6. The teneral flies were fed on infected animals during three consecutive days (day 2, 3 and 4). For each of the test series controls were run simultaneously. After the initial experimental period, all flies were fed through membranes on heparinized cow blood (glucose and ATP), kept (25-30 per cage) at 24-26°C and 75-85% RH, and their survival monitored. Samples of T. vivax infected flies were dissected starting day 10 whereas those infected with T. congolense or T. b. brucei were dissected from day 20 or 25 onwards.

Table 1. Emergence data for Glossina palpalis gambiensis pupae after combined radiation and low temperature treatment

Series	Treatment	Emergence: Pupae N	Emergence: Total %	Emergence: Male %	Average Pupal period (days): Females	Average Pupal period (days): Males
A	Control	3,444	95.6	53.4	28.8	31.2
B	D20-25 (15°C)	3,701	95.5	50.0	(32.7-35.8)	(34.6-37.8)
C	D25/60 Gy	3,189	91.2	47.5	(29.1-32.1)	(30.1-33.0)
D	D28/60 Gy	2,969	92.6	50.8	(27.8-29.0)	(29.9-31.3)
E	D28/80 Gy	3,146	91.9	48.6	(27.8-31.1)	(29.9-33.2)
F	D30/80 Gy	3,012	91.2	49.0	(*)	(29.8-31.3)
H	D30/100 Gy	2,216	92.6	49.6	(*)	29.1*
I	15°C/D20-25 D25/60 Gy	2,596	83.2	47.9	(32.8-34.1)	(34.5-36.2)
J	15°C/D20-25 D28/60 Gy	2,651	87.5	49.6	(32.5-34.0)	(34.3-36.2)
K	15°C/D20-25 D28/80 Gy	2,504	92.4	52.2	(32.8-33.9)	(34.5-36.2)
L	D28/80 Gy 15°C/D28	2,646	82.9	50.3	(29.0-32.6)	(34.4-35.8)
M	D30/80 Gy 15°C/D30	2,691	84.0	45.1	(*)	(30.8-33.9)
N	D30/100 Gy 15°C/D30	1,920	80.5	48.0	(*)	30.8

(*) Most flies emerged before second part of treatment was given; D = days following emergence

Low temparature = 15°C
Radiation treatment = 60, 80 or 100 Gy (1 Gy = 100 rad)

Relevant information on the rate of development of different trypanosome species in some of the G. p. gambiensis treatment groups can be summarized as follows:

1. For male flies in the control groups which had received the infected blood meals either as tenerals or non-tenerals:

T. vivax (Labrum and hypopharynx)(%)	98.9	and	97.9
T. congolense (Midgut/Labrum)(%)	77.5/ 1.2	and	34.7/ 1.3
T. b. brucei (Midgut/S. glands)(%)	91.1/25.3	and	54.7/10.7

2. When males had experienced in the pupal stage a temperature of 15°C for 5 days, the corresponding average infection rates (%) were:

T. vivax	(No data for teneral group)	and	87.8
T. congolense	71.3/ 2.3	and	54.2/ 7.1
T. b. brucei	77.0/12.7	and	44.9/ 6.4

3. For males which had received a radiation treatment of 100 Gy in the terminal pupal stage (day 30):

T. vivax	(No data for teneral group)	and	71.2
T. congolense	58.2/10.1	and	64.8/18.5
T. b. brucei	35.7/17.8	and	34.1/15.8

4. After a combined low temperature treatment (15°C during day 20-25 of pupal period) and radiation treatment (80 or 100 Gy on day 30):

T. vivax	96.8	and	95.7
T. congolense	No data		
T. b. brucei	62.7/26.9	and	44.4/18.0

In general, rather high midgut and salivary gland infection rates were found in G. p. gambiensis. A possible effect from adding glucose to blood used underneath the membrane requires further investigation.

EFFECT OF IRRADIATION ON TSETSE FLIES
(Summary)

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We have investigated the effects of irradiation on Glossina species which have been the subject of sterile male release campaigns in West Africa. From these sudies it is clear that (i) irradiation at standard doses used in SIT causes substantive damage to Glossina midgut and markedly changes peritrophic membrane structure, (ii) irradiation effects reduce levels of lectins and lysin secretion by Glossina midgut cells, (iii) irradiation inhibits production of midgut proteases and (iv) these changes create conditions for enhanced growth of trypanosomes in the midgut.

In addition, major differences in the biochemical profile of G. palpalis palpalis and G. p. gambiensis have been found particularly in midgut lectins and lysins and in the characteristics of salivary gland binding using labelled lectins. It is clear that G. p. palpalis (from the Vienna in vitro fed stock colony) is refractory to Trypanozoon and that its contribution to the transmission of trypanosomes (Nannomonas and Trypanozoon) in areas where this subspecies has been released is probably negligible. This study highlights the need to assess vectorial capacity of target species precisely and evaluate cost-effectiveness of any inputs in relation to control benefits.

STUDY OF TSETSE–HOST IMMUNITY RELATIONSHIPS WITH RESPECT TO MASS REARING AND TSETSE ERADICATION

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Abstract

The influence of repeated exposition of alternative hosts (rabbits, mice) to flies was characterized by two different types of adaptive response mechanisms:

i) the induction of serum "killing factor", and

ii) the induction of high titre of circulating IgG cross-reacting with relatively wide spectrum of tsetse (glyco)proteins isolated either from various tissues, or whole body homogenates.

The question remains how these pathways are involved in host-vector interaction under natural conditions. "Killing factor" seems to be responsible for dramatic increase of fly mortality following sucking. It is passively transferable but its effect disappears during about 5 days following sucking or passive immunization. On the contrary, the immunoglobulins persist in host blood for at least 70 days but they are probably not responsible for direct killing of biting flies. However, their role in fly longevity, reproduction, and vector-parasite interaction was determined.

1. INTRODUCTION

Repeated bites of tsetse flies on experimental hosts induce high titres of circulating antibodies (Brown and Cipriano,1985; Parker,1979; Ellis et al. 1986; Maťha et al.1988). Despite their high titre and relatively long time of persistance in blood (Maťha et al. 1988), their exact role in host resistance against vector (or parasite) remains still obscure, and the ideas about their role in pathogenic/deffence processes differ author from author.

Parker (1979), and Brown and Cipriano (1985) showed repeated feeding of *Glossina morsitans* on experimental laboratory animals to be accompanied by increased mortality of sucking flies and the induction of relatively high titres of circulating antibodies that showed crossreactivity with salivary gland homogenate. Similar results were obtained during experiments with *Glossina palpalis palpalis* feeding in vivo on rabbits in our laboratory (Maťha et al. 1986). However, the direct relationship between actual titre of induced antibodies and killing of sucking flies remained the subject of speculation, since repeated feeding apparently increases the titre of antibodies but neither the titre itself nor the time of antibodies' persistance in host blood exhibit a direct correlation with mortality of feeding flies (Maťha et al.1988). Consequently, the mortality seems to be affected by some factors in which circulating antibodies do not play substantial role (Maťha et al.1988). In order to investigate the role of anti-*Glossina* antibodies in host deffence and/or vector biology we attemped to characterize Ab, e.g. their persistance, specificity and direct influence on tsetse fly longevity. This report summarizes our results.

2. MATERIAL AND METHODS

Adult tsetse flies *Glossina palpalis palpalis/* (Rob.-Desv.) of Nigerian origin obtained as puparia from the stock colony of the Entomology Unit of Join FAO/IAEA Divission, Siebersdorf, Austria, were maintained under standard conditions on rabbits. Adults of *G. morsitans, G. pallidipes*, and *G. fusca* were used for antibody (Ab) specifity studies.

To study the dynamics of circulating anti-tsetse antibodies in peripheral blood experimental rabbits were exposed to two *Glossina* sucking cycles during 75 days. Rabbits were exposed untill the symptomes of induced "killing factor" were not apparent. Persistance of "killing factor" was tested by bioassay using groups of ten flies. Flies were fed in two days intervals on each immunized host and the presence of "kiling factor" was expressed as a percentage of affected flies. Antigens for the study of anti-*Glossina* antibodies in rabbit serum were prepared from freshly dissected salivary glands in ELISA coating buffer (0.1 M sodium bicarbonate, pH 9.6), centrifuged for 10 min at 5,000 g and 4 °C. The supernatant diluted to containe 10 μg of proteins per 1 ml of buffer (Lowry et al., 1951) was used in ELISA tests. For the detection of Ab the standard ELISA tests according to Voller et al. (1979). Serum from exposed rabbits was serially diluted in the range from 1 : 100 to 1 : 25,600 and incubated for 2 hrs at 37 °C. After washing with ELISA buffer the conjugate (swine anti-rabbit IgG, peroxidase stained, SEVAC Prague, diluted 1 : 1,000) was added and incubated for 2 hrs at 37 °C, washed, developed and read on MR 580 microplate reader. Intensity of reaction was recorded as the absorbance at 492 nm. Each serum sample was tested twice.

Three chinchilla rabbits weighing approximately 2 kg each were used for the passive immunity transfer experiments. They were injected twice with 15 ml of immune serum, 24 hrs apart, into the marginal ear vein. The titre of Ab of the serum of recipients and donors was measured by ELISA at the beginning of the experiment (see day -2, Table 1), after first (see day -1, Table 1), and second injection of Ab (see day 0, Table 1), and then every 24 hrs during the following week. The presence of "killing factor" was monitored

by bioassay. Transferred immunity was expressed as the corrected mortality of sucking flies.

Affinity chromatography on purified antibodies and Con A Sepharose was performed as mentioned earlier (Maťha et al., 1986). Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was used for antigen (glyco)protein separation. SDS-PAGE was performed in 5-20% gradient running gel according to Laemmli (1970) under reduced conditions. The samples were double applied on the surface of the gel and electrophoretized on Pharmacia GE 2/4 vertical slab electrophoresis apparatus under constant voltage of 120 V until the tracking dye reached the bottom of the gel. Proteins were detected by staining with Coomassie brilliant blue R-250 and/or counterstained by silver. Pharmacia low molecular calibration kit was used to determine the molecular weights of separated proteins.

For the screening detection of antibody-antigen reaction the standard immunodiffusion method by Ouchterlony (1967) in 0.8% agarose (w/v) in 50 mM Tris-HCl buffer pH 7.4 was used. The precipitation reaction was allowed 24 hrs at room temperature in a wet chamber, then the agarose gels were thoroughly washed with saline and distilled water to remove unprecipited proteins, dried, and stained by Coomassie brilliant blue. For further antigen detection and characterization, the blotting method according to Towbin et al. (1979) was used. After the electrophoresis the gels were immersed in blotting buffer (0.025 M Tris, 0.192 M glycine, 20% methanol, and 0.04% SDS) for 30 min. and the proteins were electroblotted to nitrocellulose membrane. After this procedure the NC sheet was immediately immersed in 5% skim milk in phosphate buffered saline (PBS) containing 0.05% Tween-20 for 30 min, then five times washed with PBS-Tween and incubated with horseradish peroxidase-conjugated swine anti-rabbit immunoglobulins at a dilution of 1 : 1,000 for 45 min. Thereafter the blots were washed as before and the antigen-antibody complex visualized by incubating in substrate solution (12.5 mg of 3,3-diaminobenzidine, 15 μl of 30% H_2O_2 in 50 ml of 0.1 M Tris-HCl buffer pH 7.6). The procedure was stopped by 0.5% NaN_3 after a good contrast of zones was reached. Same technique was used to visualize antigens in semithin section of *Glossina* salivary glands embedded in LR White (Polysciences).

For the statistical analysis of results the chi-square calculation and Student's t-test were used.

3. RESULTS

In order to determine the effects of host humoral immunity (mainly circulating antibodies) on the mortality of feeding flies, we have attempted to passively imunize a group of experiemental rabbits with serum of donors showing typical "killing effect" on flies. As it is apparent from Table 1, the titre of circulating antibodies in serum of recipient animals reached about 1/3 of the donor values after the first intravenous application and about 2/3 of these values after the second injection.

The results of these experiments showed that the "killing factor" which was characterized by a direct mortality of flies could be tranferred to recepient animals by passive immunization (intraveneous injection). As it is apparent from data in Table 1, only a partial defence ability can be induced by injection of immune serum into the acceptor animals. While the titres

of antibodies in the blood of passively immunized hosts persisted at almost the same level during the duration of the experiment, the partial fly-killing ability had completely disappeared within 72 hrs following injections (Maťha et al.,1989).

The results obtained are in good agreement with our earlier conlusions based solely on the kinetics of antibodies induction in experimental hosts during two feeding cycles of tsetse flies (Figure 1) (Maťha et al.,1988).

Table 1 The correlation between the titre of antibodies and induced mortality of tsetse flies fed on passively immunized rabbits

Day	Antibodies (expressed as A 492 in ELISA)			Corrected mortality (%)		
	1	2	3	1	2	3
-2	0.274	0.176	0.195	0	0	0
-1	0.462	0.403	0.436	NT	NT	NT
0	0.634	0.550	0.620	NT	NT	NT
1	0.792	0.722	0.756	15	8	12
2	0.770	0.690	0.720	10	5	10
3	0.765	0.685	0.720	10	5	8
4	0.756	0.680	0.698	5	0	2
7	0.738	0.670	0.680	0	0	0

1, 2, 3 - experimental acceptors
The A 492 of donor immune serum was 1.150
NT - not tested

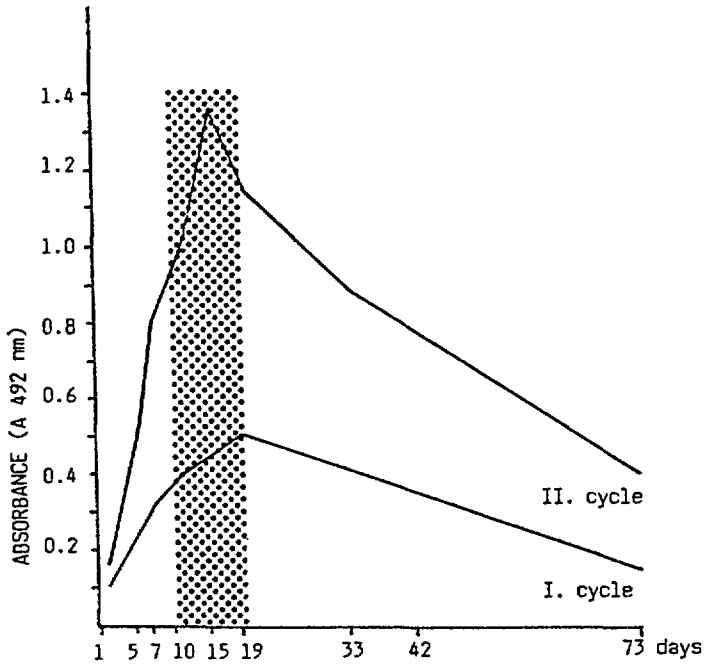

Figure 1 Comparison of the kinetics of antibody formation in serum of rabbits exposed to two subsequent biting cycles of *Glossina palpalis palpalis*. Dotted column illustrates the period during which "killing factor" was detectable in exposed rabbits during both cycles. (Titre of antibodies expressed as A 492).

From these data it is evident that antibodies against tsetse salivary gland antigens remain present in the immunized rabbits for a period of at least 70 days whereas the "killing factor" is active for a limitted period only (dotted area in Figure 1). Despite some differences in antibody titre in individual rabbits, due probably to genetic and/or physiological features in experimental rabbits, the kinetics of the formation of antibodies against tsetse salivary gland antigen(s) was at comparable level.

From the comparison of obtained results of both experiments it seems to be evident that the "killing factor" is not a constant share of the antibodies, it does not depend on the threshold of their titre only, and is not present everytime this threshold is reached.

Despite the fact the antibodies are probably not responsible for the direct killing of flies they could represent a potential tool for the study of tsetse biology since, according to literature, one could expect their importance in vector-pathogen-host relationships. Kaaya and Alemu (1982, 1984) described a significantly decreased fecundity of *Glossina morsitans morsitans* fed for 45 days on rabbits immunized with salivary glands, bovine trypsin, thoracic muscles, brain and thoracic nerve ganglia, gravid uterus proteins, and bacteria from unfed tsetse midgut. Nogge (1978) reported decreased reproductive rate in flies fed on rabbits immunized with gut mycetome endosymbionts. Similarly, Brown and Cipriano (1985) reported a reduction of feeding time together with increased mortality after repeated feeding of *Glossina morsitans* on guinea pigs. All these results reached at different time and in different laboratories show that some biological effect of Ab exists. The fact that the immunization of host with various tissues as well as endosymbionts of tsetse can induce similar biological response turned our interest towards characterization of tsetse antigens in detail.

For the isolation of antigens from tsetse salivary glands two basic techniques were used. By using of affinity chromatography on CNBr-activated Sepharose 6B with coupled rabbit IgG a single asymmetric protein peak was obtained (Figure 2).

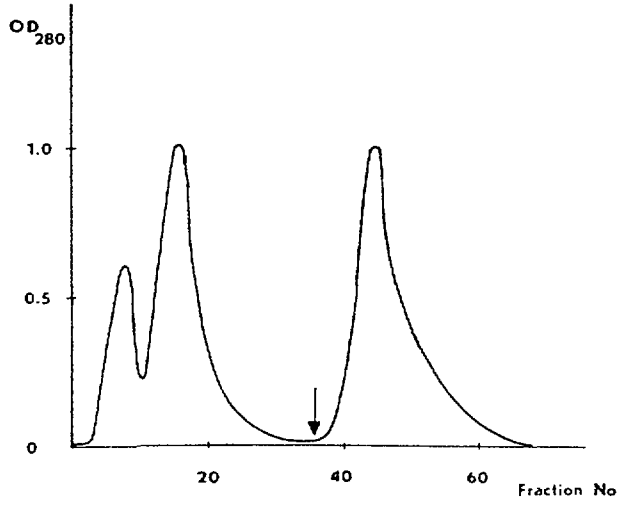

Figure 2 Isolation of salivary gland antigens by using of affinity chromatography on IgG coupled CNBr-activated Sepharose 6B. Arrow indicates the application of 0.1 M glycine buffer pH 2.5.

Its asymmetry suggests that several proteins could be present in this fraction. SDS-PAGE divided this cluster into four protein fraction. Of these, two major fractions have respective molecular weight of 55 (I) and 60 (II)kD, respectively. Minor fractions visualized by silver staining only exhibited m.w. of 160 (III), and 200(IV) kD, respectively (Figure 3, fractions I-IV).

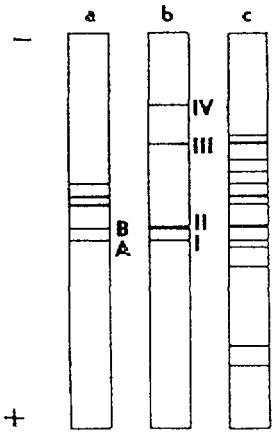

Figure 3 Zymograms of glycoproteins isolated on Con A Sepharose (a), antigen isolated on IgG couled CNBr-activated Sepharose 6B (b), and of whole salivary gland homogenate (c) in 8% running SDS-PAGE gels according to Laemmli (1970).

Separation of salivary gland homogenates on Con A Sepharose column demonstrated presence of four glycoprotein peaks (Figure 4, peaks a,b,c,d).

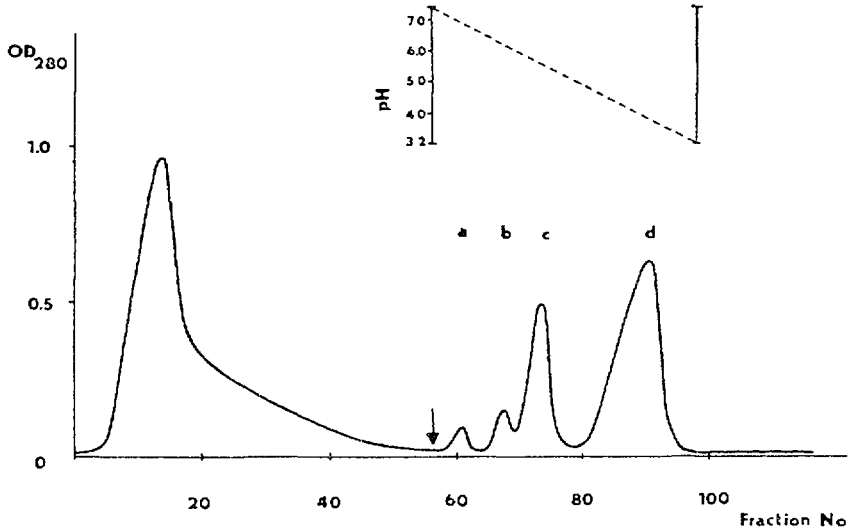

Figure 4 Affinity chromatography of salivary gland homogenate on Con A Sepharose. Arrow indicates the beginning of pH gradient application. a,b,c, and d - individual glycoprotein peaks.

(Glyco)protein patterns of individual peaks are apparent from Figure 5.

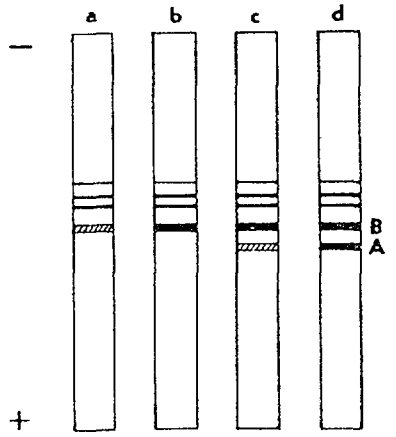

Figure 5 Differences in patterns of individual glycoprotein peaks eluted by pH gradient from Con A Sepharose column. a,b,c, and d - individual peaks as in Fig. 4. A and B - glycoprotein fractions exhibiting a positive reaction with the serum of exposed rabbits in counter current immunoelectrophoresis.

The comparison of figure 3a and 3b suggests that, based on m.w., the glycoprotein fractions A and B (Figure 3a) could be considered as indentical with major fractions I and II obtained by affinity chromatography on IgG coupled Sepharose 4B. These conclusions were further supported by the results of counter current immunoelectrophoresis. Glycoproteins contained in concentrated fractions A and B exhibited the same positive reaction with the serum of exposed rabbits as fractions I and II of Fig. 3b did (for details see Maťha et al.,1986).

Despite this apparent success in semipurification and partial identification of antigenic (glyco)proteins, a proper explanation of the fact why different tissues can iduce similar response was not obtained. The idea we have followed in further studies was the existence of common epitope(s) which are sharing by different tissues of the fly. To test this possibility the immunodiffusion test according to Ouchterlony was used. By using of this technique, common antigens to salivary glands, mesenteron, fat body, thoracic muscle, and whole body homogenates of native and dried tsetse flies, *Glossina palpalis palpalis* were demonstrated (Maťha and Weiser,1988).

For further characterization of antigenic cross-reactivity between individual tissues homogenates, the western blotting according to Towbin et al. (1979) was used. As it is evident from Figure 6 sera from hyperimmunized rabbits were shown to react with at least 20 protein fractions in salivary gland homogenates. M.w. of them ranged from 15 to 160 kD.

Figure 6 Western blot antigen profile of *G. palpalis palpalis* native whole body homogenate (1), dried whole body homogenate (2), salivary gland (3), fat body (4), thoracic muscle (5), and mesenteron (6) homogenates. The blot was stained using an immunoperoxidase protocol, with pooled hyperimmune rabbit serum as the first antibody. The empty line (control) was stained using normal rabbit serum. Pharmacia LMW calibration kit was used to detremine m.w. of separated proteins (numbers of left margin= kD of calibration proteins).

In addition 6 common antigens(epitopes) were detected in all homogenates tested. These fractions could represent a group of common epitopes shared by different tissues of tsetse fly. The specificity in their distribution was tested by using salivary glands or whole body homogenates of other *Glossina* species. Results are summarized in Figure 7.

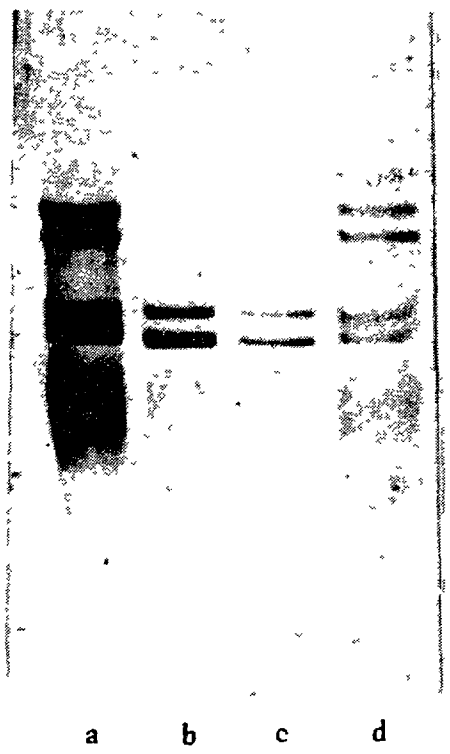

Figure 7 Western blot cross-reactivity profile between homogenates of *G. palpalis palpalis* (a), *G. morsitans* (b) *G. pallidipes* (c), and *G. fusca* (d). The blot was stained using anti *G. palpalis palpalis* hyperimmune rabbit serum as first antibody.

How it is evident from the picture, antibodies developed during the sucking against *G. palpalis palpalis* saliva could recognize several common protein bands in homogenates of *G. morsitans, G. pallidipes*, and *G. fusca.* Moreover, antibodies against *Glossina palpalis palpalis* could cross-react with two major fractions (m.w. of 53 and 62 kD), and four minor (m.w. of 28, 30, 32, and 49 kD) fractions of SDS-PAGE separated lysate of procyclic form of *T. brucei* (Maťha and Grubhoffer,1988).

4. DISCUSSION

How it is evident from our results, the direct involvement of circulating antibodies in fly killing was not confirmed. According to our latest experiments the induction of "killing factor" is accompanied rather by some behavioural or ethological changes of flies than by antibodies themselves (Volf et al., unpubl.) It is possible that bioactive polyamines which are realized from host blood cells due to repeated intradermal hypersensitization by salivary gland antigens could be involved in the interference with *Glossina* physiological or, rather behavioral functions. Experiments evaluating this idea are going on in our laboratory now. Despite the lack of indentification of direct involvement of atibodies in tsetse killing, they could represent an important tool in studies of *Glossina* physiology.

The fact that different tissues bear common antigens or epitopes can represent an important step in vector-parasite relationships studies. Nowadays only limited data are available on the interaction between vector immunity and trypanosomes. Croft, East and Molyneux (1982) and East, Molyneux, Maudlin and Dukes (1983) have demonstrated that in *Glossina* species there is an anti-trypanosomal factor in the hemolymph which immobilizes the procyclic forms of *T. brucei* and *T. congolense* and bloodstream forms of *T. congolense* and *T. vivax* but not the related kinetoplastid flagellates *L. hertigi* and *C. fasciculata*. However, little immunological studies have been done on the response of tsetse to parasites. We believe, that vector immunology represents a field where anti-tsetse antibodies could be used. According to our results, relatively broad cross-reactivity of antibodies exists in the range of *Glossina* genus. On the contrary no cross-reactivity was determined by ELISA and western blotting with other diptera (black flies, mosquitos, house fly) or invertebrate (various parasitic worms) homogenates (Lukeš et al., unpubl.). These results show on the existence of some selective recognition of *Glossina* antigens (or rather epitopes). Similar results were obtained with trypanosomes. Several bands cross-reacting with rabbit anti-*G. palpalis palpalis* antibodies were detected in the lysate of procyclic forms of *T. brucei*. And moreover, living procyclic forms of *T. brucei* treated by the same rabbit serum were immobilized during 20 min. These our results are comparable with those of East et al. (1983) describing similar effect of anti-trypanosomal factor in tsetse hemolymph. It seems probable that antibodies induced during the sucking can recognize some "common *Glossina* antigens". We suppose that the existance of such antigens (epitopes) which could be shared both by vector, both by parasite, could help the trypanosome to escape vector's recognition and defence reactions. So, from the point of vector-parasite immunobiology, the antibodies induced during the natural sucking process, with their high specificity against "common *Glossina* antigens", could represent a new useful approach to these studies.

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Part IV

TSETSE FLY CONTROL, TSETSE GENETICS AND TSETSE REARING

THE DURABILITY OF PYRIPROXYFEN, A JUVENILE HORMONE MIMIC, FOR INSECT CONTROL

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Abstract

Topical applications of the juvenile hormone mimic, pyriproxyfen, to adult females of the tsetse fly, Glossina morsitans morsitans, result in the production of offspring which fail to metamorphose. Similar applications to adult females of the triatomine bug Rhodnius prolixus, result in the production of non-viable eggs, while treatment of fifth instar larvae prevents metamorhposis or gives rise to adults which produce non-viable eggs.

Exposure by tarsal contact to netting surfaces treated with an oil formulation of radiolabelled puriproxyfen, resulted in adult females of Glossina m. morsitans producing non-viable pupae for life. Netting stored under laboratory conditions showed no sign of loss or degradation of pyriproxyfen for 8 months. Similar netting exposed to natural conditions in the field in Zimbabwe has similarly shown no signs of degradation or of loss of the original amount of pyrifproxyfen with which it was treated.

The prospects of using pyriproxyfen as a safe substitute for conventional insecticides are promising.

Introduction

The failure of tsetse flies to develop genetically dependent resistance to insecticides can in part be attributed to their low rate of reproduction, producing fewer generations per year than most egg-laying insects and therefore fewer opportunities for genetic selection to occur. However, there are probably other equally important reasons for this lack of development of resistance. Firstly, sub-lethal doses of pyrethroids especially, induce temporary knockdown. This means that under field conditions the insect will either be eaten by ants or will die from overheating on the ground. Hence, there is little opportunity for a tsetse to survive a sub-lethal dose of insecticide. Furthermore, the distribution of tsetse and the discontinuity both spatially and temporally, of insecticide campaigns against tsetse, means that populations recover largely by re-invasion from neighbouring areas and from adults emerging from puparia protected underground, neither of which have come into contact with the insecticide used in the campaign.

Nevertheless, as more countries begin to use insecticides for tsetse control, the opportunity for individuals to survive sub-lethal doses must increase. Therefore, there is every reason to encourage the simultaneous use of insecticides with different metabolic effects upon the target insect, in order to minimise the risk of selection for resistance.

At present, a great deal of competitive effort is being directed towards producing pyrethroids with ever increasing potency and durability. Such compounds are all generically the same and act as neurotoxins. Hence, selection for resistance to one will probably suffice for all.

The juvenile hormones are attractive as complements to pyrethroids in that they act to disrupt embryogenesis and metamorphosis (Langley & Pimley 1986). Their value in tsetse control programmes will depend upon formulations which facilitate their use in conjunction with odour-baited traps and targets. Odour-baited targets treated with pyrethroids have already been used successfully to control tsetse populations (Vale et al. 1985, 1986, 1988).

In addition to the theoretical advantages of transfer of juvenile hormones from males to females during mating (Langley & Weidhaas 1986), it is essential to demonstrate that such compounds are highly durable under field conditions. This is because a major portion of the cost of a tsetse control operation involving insecticide treated targets, is the re-treatment of the targets.

Pyriproxyfen has been shown to be a highly effective juvenile hormone mimic for the disruption of reproduction in tsetse both in the laboratory (Langley et al. 1988, 1990) and in the field (Hargrove & Langley 1990). Its value for tsetse control will depend upon ints durability. Hence, the present report covers an investigation of the persistence of this compound under natural conditions in the field and a study of its fate once it enters the target organism.

Methods

Insects

Adult females of Glossina morsitans morsitans and Rhodnius prolixus were obtained from colonies maintained at TRL at 25°C and 60-70% r.h. and fed routinely on defibrinated pig blood through silicone rubber membranes (Mews et al. 1977, Langley & Pimley 1978).

Treatment of insects

Adult females of G. m. morsitans at the start of their second reproductive cycle were dosed topically on the ventral abdomen with 1 ul acetone containing 10 ug pyriproxyfen plus 2 x 10^5 dpm ^{14}C pyriproxyfen. Recently moulted adult females of R. prolixus were similarly treated but received 3 x 10^5 dpm labelled pyriproxyfen.

Maintenance of insects and sampling techniques

Treated Rhodnius females were housed with equal numbers of males, in netting covered, clear polystyrene cups containing filter paper perches. Eggs were harvested and the insects offered food weekly. Sub-samples of weekly egg collections were subjected immediately to scintillation counting and the rest were stored in methanol in glass vials for HPLC analysis. Other eggs, collected during the week following treatment of the adult females, were incubated at 25°C and sampled weekly until the time when normal eclosion was

expected (21 days). Again, sub-samples of each collection were subjected to scintillation counting and the rest were stored in methanol for HPLC analysis. Thirty days after treatment, some eggs began to hatch. Therefore, both non-viable eggs and recently hatched first instar larvae were sampled and analysed.

Females of G. m. morsitans were housed in groups of 10 in circular plastic cages and offered food daily. Puparia were harvested and assigned to the appropriate reproductive cycle following treatment of the adult. Puparia produced during the cycle immediately following treatment were incubated at 25°C and sampled at intervals during their development. Some were subjected to scintillation counting, while others were stored in methanol for HPLC analysis. Puparia from the second and third reproductive cycles following treatment were also sampled but were not incubated beyond 10 days.

Adult females of both G. m. morsitans (36 days after treatment) and R. prolixus (14 and 53 days after treatment) were analysed by scintillation counting and HPLC, as were untreated R. prolixus males that had spent the whole of the experimental period with their mates.

Treatment of netting surfaces with pyriproxyfen

Samples of black terylene netting (of the type used as invisible flanking nets on odour-baited targets treated with pyrethroids) were immersed in cereclor ^{R} /acetone mixtures containing pyriproxyfen, such that after removal and evaporation of the acetone, a residue of 7.5 ul oil and 0.1 mg pyriproxyfen containing 1.0 x 10^5 dpm cm^{-2} was left on the netting surface (laboratory exposed netting). Netting samples prepared for exposure in the field contained the same quantity of oil and pyriproxyfen, but only 1.5 x 10^4 dpm radiolabel.

Each netting sample measured 5 cm x 2 cm. A series of samples was prepared and exposed in the laboratory at TRL, while a similar series, prepared in the same way was exposed to the natural environment in the Zambezi Valley, Zimbabwe. Each month, samples of the material, dispatched in aluminium foil, were subjected to scintillation counting and to HPLC analysis. Samples were then tested at TRL for their ability to induce sterility in G. m. morsitans females at the start of their second reproductive cycle by tarsal contact exposure either briefly, for 2 seconds or for 5 seconds. Treated females were kept for 45 days and the viability of their offspring was monitored.

Analytical Techniques

Radioactivity measurements

Insects were homogenised in methanol and centrifuged. Homogenates were washed three times with methanol and washings added to the original extract. After evaporation of the methanol in a plastic scintillation vial, 3 ml of Optiphase "HiSafe" II (LKB) scintillant was added and radioactivity measured in a LKB Wallac 1217 Rackbeta Scintillation Counter. Netting samples were immersed in 3 ml scintillant and counted directly.

Analytical HPLC Method

Samples were sonicated for 2 minutes in 2 ml CH_3CN. Five hundred microlitres of the solution were transferred to an Eppendorf tube and centrifuged at 200 rpm for 10 minutes. A 20 ul sample of the supernatant was used for HPLC analysis using reverse phase chromatography on an 8 x 100 mm Nova-Pak C18 cartridge (Waters) packed with 4 um packing material, using 60%

CH_3CN in water (60/40; v/v). A Waters 600E system controller was used with a flow rate of 1 ml min^{-1} and UV absorption of eluting compounds was monitored with a multi-wavelength photodiode array detector (Waters 990), with plotter setting at 220 nm. Fractions were collected at one minute intervals for quantification of ^{14}C using an LKB scintillation spectrometer.

Results and Discussion

The fate of pyriproxyfen in R. prolixus

Results are summarised in Table 1 from which it is concluded that 10% of the pyriproxyfen administered topically, remained in an adult female for 14 days during which time between 0.1% and 0.5% of that administered appeared in each of her eggs. There was no indication that pyriproxyfen was metabolised within the egg even after 21 days development and eggs laid up to 28 days after treatment of the adult females still contained a high percentage of authentic pyriproxyfen. However, eggs produced more than 30 days after treatment of the adult females, contained a much lower percentage of authentic pyriproxyfen (<30%) which was not reflected in a similar reduction in the adult themselves which contained over 70% authentic pyriproxyfen even 53 days after treatment.

This is a strong indication that as metabolism of pyriproxyfen proceeds in the female, a higher proportion of the polar metabolites is channelled into the eggs at the expense of the authentic pyripoxyfen which appears to be retained in the body of the female.

There was a gradual decrease in the amount of pyriproxyfen transferred to eggs from about 50 ng (1.7×10^3 dpm) during the first week following treatment, to around 2.5 ng (0.8×10^2 dpm) between 21 and 28 days after treatment. This, coupled with the reduction in the amount of authentic pyriproxyfen to less than 30% resulted in eggs laid more than 30 days after treatment being viable (Table 1).

Of particular interest was the observation that male bugs housed with treated females became radioactive, each containing around 25 ng of which only 6.5% was authentic pyriproxyfen. Therefore, unless the males have an enhanced capability to metabolise pyriproxyfen compared to females, it seems likely that they became contaminated through contact with faecal matter in the cages.

The fate of pyriproxyfen in G. m. morsitans

A surprisingly large proportion of the pyriproxyfen administered to an adult female at the start of her second reproductive cycle was transferred to her larva, approximately 20% being detected in the puparia produced in the cycle following treatment (Table 2). Of this amount, around 90% was authentic pyriproxyfen for the first three days of pupal life. Thereafter, the amount of authentic pyriproxyfen fell to about 80%, probably reflecting the ability of the adult to metabolise the compound.

Table 1: The fate of pyriproxyfen in adults and eggs of *R. prolixus* after treatment of adult females topically with with 1 ul acetone containing 10 ug pyriproxyfen and 3 x 105 dpm radiolabelled pyriproxyfen before placing with males.

Sample	Time after treatment (days)	Age of sample (days)	Radioactivity dpm	ng equiv.	Authentic pyriproxyfen (%)
Egg	<7	1 - 7	$2.2x10^2$	7	94.5
Egg	<7	7 - 14	$1.7x10^3$	57	92.0
Egg	<7	14 - 21	$4.5x10^2$	15	92.0
Egg	7 - 14	1 - 7	$2.0x10^2$	7	89.2
Egg	14 - 21	1 - 7	$3.4x10^2$	11	82.3
Egg	21 - 28	1 - 7	$0.8x10^2$	3	91.7
Egg	>30	1 - 25	$<1.0x10^2$	<3	22.0
1st instar	>30	>20	$<1.0x10^2$	<3	27.0
Adults					
Female	14	-	$2.2x10^4$	730	79.5
Female	53	-	$7.0x10^3$	233	73.0
Male	53	-	$8.0x10^2$	27	6.5

Table 2: The fate of pyriproxyfen in adults and puparia of *G.m.morsitans* after treatment of adult females topically with 1 ul acetone containing 10 ug pyriproxyfen and 2 x 10^5 dpm radiolabelled pyriproxyfen

Reproductive cycle after treatment	Age of puparia (days)	Radioactivity/insect dpm	ug equiv.	Authentic pyriproxyfen (%)
1	1 - 3	$4.0x10^4$	2.0	98.6
1	1 - 3	$3.4x10^4$	1.7	87.2
1	10 -13	$3.7x10^4$	1.8	88.4
1	17 -20	$4.0x10^4$	2.0	81.6
2	1	$1.0x10^4$	0.5	83.0
2	7 -10	$5.0x10^3$	0.25	86.2
3	1 - 6	$6.0x10^2$	0.03	65.0
End of 3 (36 days)	Adult female	$6.0x10^3$	0.30	77.0

Puparia produced in the second cycle following treatment of their mothers contained less than 5% of that administered but still more than 80% was authentic pyriproxyfen.

In the third cycle after treatment only 0.3% of the amount administered appeared in the puparium and the proportion of authentic pyriproxyfen fell to 65%. At this time, the adult female contained 3% of the amount administered and this was 77% authentic pyriproxyfen. Therefore, it seems that the adult female tsetse metabolises pyriproxyfen only slowly and that the proportion of authentic material in puparia reflects that in the mother. There is little indication that the compound was metabolised further in the puparium itself.

Stability of Pyriproxyfen on Netting

Over a period of several months, the treated netting kept in the natural environment faded in colour and accumulated a light covering of dust. Analysis by HPLC showed that all the material present on the cloth was authentic pyriproxyfen. Results summarised in Table 3 indicate that the total amount of material on the cloth fell to less than 20% of the original after 8 months, although 75% remained for at least 5 months. Such losses did not occur in the laboratory.

Table 3: Stability of pyriproxyfen on treated netting surfaces as indicated by radioactivity measurements and HPLC analysis.

Experimental conditions	Age of netting (months)	Radioactivity dpm cm^{-2}	ug	Authentic pyriproxyfen (%)
Laboratory	0	1.0×10^5	100	100
	7	$3\text{-}4 \times 10^4$	35	100
	10	4.4×10^4	44	100
	12	7.0×10^4	70	100
Field	0	1.5×10^4	100	100
	4	1.05×10^4	70	100
	5	1.06×10^4	70	100
	8	2.2×10^3	15	100
	9	2.52×10^3	17	100

General Conclusion

Previous research has shown that only 20 ng pyriproxyfen is needed to sterilize a tsetse female for life (Langley et al. 1990). The rate at which she metabolises the compound is low and even though it is eliminated from the body, sufficient remains to ensure that all offspring produced fail to complete metamorphosis.

The pattern of metabolism and elimination of pyriproxyfen is similar in the R. prolixus female. However, insufficient material is transferred to eggs to prevent their development after about 30 days following treatment of the adult with a massive dose (10 ug). Nevertheless, the strategy for controlling this insect with pyriproxyfen will probably be to ensure multiple exposures to the material by treating resting areas such as cracks and crevices in buildings.

The inherent stability of pyriproxyfen and the extreme sensitivity of the tsetse larva to very small doses suggests that the compound could be a useful complement to pyrethroids for use with odour-baited targets for tsetse control.

Increasing the amount of pyriproxyfen used to treat a target before deploying it in the field could easily compensate for the recorded losses and the present study has shown that problems associated with breakdown of insecticides exposed to natural conditions would not be a problem with pyriproxyfen.

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GENETIC STUDIES ON *Glossina morsitans* AND *Glossina palpalis* RELATED TO GENETIC CONTROL

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Abstract

Non-assortative mating of closely related taxa and their low reproductive capacity makes tsetse likely targets for population suppression by use of sterile hybrids or satyrs. Evaluation of the potential for such control methods will be facilitated by a knowledge of the genetics of tsetse flies. At the University of Alberta, such studies are carried out on the following subspecies of tsetse: G. m. morsitans, G. m. centralis, G. m. submorsitans, G. p. palpalis, and G. p. gambiensis. Most of the genetic variants are detected by electrophoretic procedures, but five visible markers (ocra, salmon and sabr in G. m. morsitans; tan and brick in G. p. palpalis) are also maintained. Fourteen marker genes have been mapped in G. m. morsitans, four in G. m. submorsitans and two in G. p. palpalis. Other biochemical markers have been assigned to autosomes or the X chromosome and inbred lines are being established for mapping four X chromosome genes and four autosomal genes in G. p. palpalis. These marker genes are being used in studies of the genetic basis of hybrid male sterility, potential for introgressive hybridization, sperm use by double-mated females, genetic stability of tsetse colonies, and genetics of mating behaviour. Using genetically marked lines it has been established that an X - Y incompatibility is a major factor in the sterility of males produced by hybridizing G. m. morsitans and G. m. centralis, G. m. submorsitans and G. m. morsitans, and G. p. palpalis and G. p. gambiensis. In subspecies of morsitans there are also maternally inherited factors that create asymmetries in the ability of flies to hybridize. Using genetically marked flies, it has been established that some twice-mated females use sperm from both matings. However sperm use is, in some cases, influenced by whether the male is from the same subspecies as the female and this suggests a severe limitation on the use of satyrs as genetic control agents.

Introduction

It has long been recognized that the low reproductive capacity of tsetse flies makes them ideal targets for control by genetic means. Although a wide variety of such methods could be employed (Whitten and Foster 1975) only two approaches have been considered for use against tsetse flies: introduction, into a natural population, of genes or chromosomes that are deleterious; and release of sterile (or effectively sterile) males. Curtis (1968a) proposed that suppression or eradication of tsetse populations could be attained by release of flies bearing translocations and he undertook preliminary laboratory experiments to establish translocation lines of G. austeni (Curtis 1969a, 1969b, 1970b, 1971; Curtis et al. 1972). I proposed the use of a deleterious allele salmon for the control of G. m. morsitans (Gooding 1982a), but abandoned plans to pursue this approach when it was discovered that flies with salmon eyes were very good vectors of trypanosomes (Makumyaviri et al. 1984, Distelmans et al. 1985).

The classical approach to eradication of insects by genetic techniques is by release of males sterilized by chemicals or irradiation. This approach has been successfully employed with G. m. morsitans in Tanzania (Williamson et al. 1983), with G. p. gambiensis in Burkina Faso (Politzar and Cuisance 1984) and G. p. palpalis in Nigeria (Olandunmade et al. 1990). The benefits and limitations of this approach to tsetse control have been reviewed elsewhere and will not be repeated here.

The possibility of controlling tsetse flies by hybrid sterile males was proposed by Vanderplank (1944) and he undertook a limited experiment in which a population of G. swynnertoni in Tanzania was apparently eradicated by release of large numbers of G. m. centralis (Vanderplank 1947). Recently Ribeiro (1988) proposed that satyrs (i.e. males that mate with heterospecific females) may be used to eradicate pest insects. He reinterpreted Vanderplank's eradication experiment as a possible example of the effects of satyrs, rather than solely the effect of sterile hybrid males. Whether the population was suppressed by the effects of satyrs or of hybridization and hybrid sterility remains a matter of speculation. In any event, satyrs and sterile hybrid males may be useful as genetic control agents, but their potential can not be evaluated without understanding the genetics of tsetse flies and the use of sperm by twice-mated females.

Since mid-1973 tsetse colonies have been maintained at the University of Alberta and since 1975 studies have been conducted on the genetics of several species of tsetse. The most extensive work has been on G. m. morsitans but recently studies of the genetics of G. m. submorsitans and G. p. palpalis have been undertaken. These studies have several objectives that pertain to tsetse control. The first is to determine the genetic basis of hybrid sterility and to determine the genetic limitations that restrict usefulness of hybrids as genetic control agents. The second objective is to establish breeding programs that produce easily identifiable sterile hybrid males. The final objective is to establish genetically marked, inbred lines for studies of genetic disequilibria, assortative mating and other aspects of mating behaviour, sperm use and sperm precedence, and the genetic changes in colonies and isolated natural populations.

Five visible markers are available in tsetse: ocra (Bolland et al. 1974), salmon (Gooding 1979) and sabr (Gooding 1984b) in G. m. morsitans; tan (D'Haeseleer et al. 1987) and brick (Abbeele and D'Haeseleer 1989) in G. p. palpalis. One physiological marker gene, Sr on the X chromosome, affects the sex ratio in G. m. submorsitans (Gooding 1986). All other marker genes are detected by electrophoretic techniques. In G. m. morsitans 14 loci have been mapped (Gooding et al. 1988, and references therein) and experiments are in progress to map two more loci. In G. m. submorsitans three biochemical marker genes and Sr have been mapped (Gooding et al. 1989) and in G. p. palpalis two genes (tan and Est-t) have been mapped on the X chromosome (D'Haeseleer et al. 1987), and four others have been assigned to the X chromosome and four loci have been assigned to the autosomal linkage groups (Gooding unpublished).

Here, I review briefly the published information on the genetic basis of hybrid sterility, and information on the probable limitations, as genetic control agents, of F_1 hybrid males and maternally inherited sterility factors . As a contribution to evaluating the potential of satyrs as genetic control agents, I present results of preliminary experiments on the use of sperm by "twice-mated" female tsetse flies. I believe that these results indicate that there may be severe limitations on the potential of satyrs as genetic agents for the suppression or eradication of some tsetse species.

Methods of studying genetics of hybrid male sterility

Genetics of hybridization in tsetse flies has been reviewed recently (Gooding 1990a) and only the salient features will be outlined here. The reader is referred to the above mentioned paper, and the references therein, for a detailed treatment of the subject.

Generally, studies of hybrid male sterility in tsetse flies have been carried out as outlined below. Most often the studies involve reciprocal crosses; males from these crosses are usually sterile, but exceptions are known. Hybrid (i.e. F_1) females are usually fertile and may be backcrossed to one or both of the parental taxa. The backcross males that are obtained are tested for fertility and then scored for marker genes to relate their genetics to fertility. Very few studies of hybrid sterility in tsetse have used flies carrying marker genes on all of their chromosomes. Furthermore, many studies have used transfer of motile sperm as the criterion for fertility. Although insemination ability may be equivalent to fertility in some cases, it is not invariably so, as can be seen in the data from crosses of subspecies of G. morsitans (Gooding 1987, 1989). Here, I refer to sterile males as those that fail to fertilize females, regardless of whether they inseminate the females. A final point concerning techniques is that to determine whether a male is fertile or sterile he should be mated with at least two females from each of the taxa being studied. The reasons for this will become apparent below.

Sterility resulting from interaction of chromosomal genes

Data have been published on only two models of tsetse hybridization in which each chromosome carried at least one marker gene: G. m. morsitans / G. m. centralis (Gooding 1987) and G. m. submorsitans / G. m. morsitans (Gooding 1989). The later model is complicated by the fact that F_1 females can be fertilized only by G. m. submorsitans. A third model (G. m. submorsitans / G. m. centralis) is now being studied in my laboratory but data on fertility and genetics of backcross males is not yet available. One model (G. p. palpalis / G. p. gambiensis) has been studied using flies carrying an X chromosome marker gene (Gooding 1988).

Data from all of the models indicate that a major cause of hybrid male sterility is an incompatibility of X and Y chromosomes from two taxa (Table 1). Nonetheless, nearly 30% of the sterile males in the morsitans / centralis model had both of their sex chromosomes from the same subspecies. These results suggest that autosomes and/or intrachromosomal recombination are involved in hybrid male sterility. However, no support for either of these possibilities has been found (Gooding 1990a). The high prevalence of fertility (ca 20%) in hybrid males obtained in the morsitans / submorsitans model has been interpreted as indicating that incompatibility of the X and Y chromosomes (or their gene products) has not evolved as far in this model as it has in the morsitans / centralis model. The availability of two X chromosome marker genes (ocra and salmon) in G. m. morsitans permits easy recognition of males carrying heterosubspecific sex chromosomes and thus the sterile males that have descended from backcrossed F_1 females. However, nearly 30% of the sterile backcross males can only be recognized by mating them with appropriate females and there is no satisfactory explanation of the genetic basis of their sterility (Gooding 1990a).

Data in Table 1 indicate certain limitations to the potential use of sterile hybrid males as genetic control agents. First, generalizations about the sterility of F_1 males may not be accurate; each model may be unique and therefore must be assessed on its own merits. Second, the data that are available are very limited and some of the variation in the prevalence of

Table 1. Fertility of hybrid and backcross males in three models of hybridization of tsetse flies.[1]

	Models Studied		
	Gmm/Gmc[2]	Gmm/Gms[3]	Gpp/Gpg[4]
F_1 males	1/101	16/81	1/34
Backcross males			
Consubspecific X/Y[5]	28/52	10/15	23/32
Heterosubspecific X/Y[6]	0/55	0/157 9/168	4/26

1 Numbers in the body of the table are: no. of fertile males/no. tested. Abbreviations of species: Gmm = G. m. morsitans; Gmc = G. m. centralis; Gms = G. m. submorsitans; Gpp = G. p. palpalis; Gpg = G. p. gambiensis.

2 Data from Gooding (1985, 1987). Males tested with G. m. morsitans females.

3 Data from Gooding (1985, 1989). Males tested with G. m. submorsitans females.

4 Data from Gooding (1988).

5 Consubspecific indicates X and Y from same subspecies.

6 Heterosubspecific indicates X and Y from different subspecies.

7 Descended from F_1 females.

8 Descended from F_1 males.

fertility among F_1 males may be due to differences attributable to choice of colonies or to geographic variation within a taxon. It will therefore be necessary to determine the level of sterility in the hybrid males from the colonies that are to be used as the source of material for any hybrid sterile male release program. Third, the low prevalence (1 to 3%) of fertile males in the morsitans / centralis model and in the gambiensis / palpalis model may be acceptable for purposes of a sterile male release program. However, the occurrence of 20% fertile males among the F_1 males in the morsitans / submorsitans model is unacceptably high. With this prevalence of fertile males it would be impossible to obtain a ratio greater than 4 sterile males to 1 fertile male in the field.

Another problem that arises from the proposed use of sterile hybrid males is that for each hybrid male produced, a fertile hybrid female is produced. These females can not be released but they could be used to produce backcross males, 75% of which are sterile. Unfortunately, with the information and genetic markers available, it is possible to recognize only about two thirds of the sterile males. Thus there is some doubt as to whether it is worth the extra investment to produce sterile backcross males.

An additional problem is the question of whether the sterile hybrid males could effectively complete with field males to effectively sterilize females in the natural population. Two questions are addressed below.

Firstly, would the laboratory-reared mutant males be as successful as males in the natural population at finding females? This question needs only arise if backcross sterile males are to be used. If only F_1 males were to be used then there is no need to use genetically marked lines and there is evidence that laboratory-reared tsetse perform as well in the field as do flies from the natural populations (Dame et al. 1975). Unfortunately, at the present time, there is no evidence that this is true for hybrids, and this would have to be established using flies from the colonies supplying the hybrid males.

Secondly, are hybrid males as effective as fertile males at inducing monogamy in females? Curtis (1972) presented some data on this question. For the morsitans / submorsitans model these data indicate that F_1 hybrid males were about as effective as parental line males at inducing monogamy. However, about 65% of the females that mated with a hybrid male and then were placed with a parental line male became inseminated (30 of 46 females were inseminated, 30 of 30 control females were inseminated, $\chi^2 = 1.49$, 1 d.f., $p > 0.05$).

Sterility induced by maternally inherited factors

Data summarized in Table 2 illustrate that among subspecies of G. morsitans there is an asymmetry in the ability of backcross males to fertilize females from parental taxa. Specifically the grandsons of G. m. morsitans that were fertilized by G. m. centralis were rarely able to fertilize G. m. centralis females (Gooding 1987). When fertilization did occur the offspring died at a young age. Similarly of the grandsons of G. m. submorsitans that were fertilized by G. m. morsitans were rarely able to fertilize G. m. morsitans (Gooding 1989). In addition to revealing an unusual inheritance pattern for hybrid male sterility, these experiments demonstrated the necessity of assessing the fertility of each backcross male by mating him with females from both parental taxa.

Table 2. Summary of published evidence for maternally inherited factors affecting unidirectional sterility in hybrid and backcross males[1].

Model taxon A / taxon B	Grandsons of	Proportion of males fertilizing Taxon A	Taxon B
centralis/morsitans	morsitans	2/372	19/37
	centralis	11/17	9/17
morsitans/submorsitans	submorsitans	2/15	10/15
	submorsitans	0/163	9/163

[1]Data, from Gooding (1987, 1989), are for males having both sex chromosomes from the same subspecies.

[2]One of two females was fertilized by each male. One female produced two larvae which failed to pupariate. The other female produced three puparia from which deformed adults emerged; all died as tenerals.

[3]These males descended from F_1 hybrid males; all other males descended from F_1 hybrid females.

Because G. m. morsitans females are rarely fertilized by G. m. submorsitans males it was not possible to test their grandsons to see if they display asymmetry in reproductive success. A further complication is that F_1 females in the submorsitans / morsitans experiment can only be backcrossed to G. m. submorsitans, and therefore it was not possible to do a complete genetic analysis of the basis of sterility. However this analysis was possible in the centralis / morsitans model and the results indicate that the inheritance pattern was strictly maternal. Since the F_1 females could be backcrossed to either parental taxon it was possible to obtain backcross males that carried only G. m. centralis marker genes but had maternally inherited factors from G. m. morsitans; these males were unable to fertilize G. m. centralis (Gooding 1987).

The apparent independence of sterility due to maternally inherited sterility factors and the origin of the chromosomes in backcross males led me to suggest that maternally inherited sterility factors might be useful as genetic control agents (Gooding 1987). The proposal was to cross G. m. morsitans females with G. m. centralis males and, by recurrent backcrossing to G. m. centralis, establish an inbred line having maternally inherited sterility factors from G. m. morsitans and virtually all of the chromosomal genes from G. m. centralis. However, when I attempted to establish such a line, recurrent backcrossing to G. m. centralis resulted in a progressive increase in the number of males that were able to fertilize G. m. centralis (Gooding 1990b). By the eighth backcross generation more than 80% of the males were fertile.

Satyrs as potential genetic control agents

For the purposes of this discussion, a satyr is a male that will mate with heterospecific or heterosubspecific females. In the simplest case the mated females behave as though they had mated with males from their own taxon, but such matings result in no offspring. When these conditions are approximated in natural, mating with satyrs adds a burden to the population to which the females belong and the population could be suppressed or even driven to extinction. Other cases with less severe burdens may still have significant effects upon the population (see the models proposed by Ribeiro and Spielman 1986, Ribeiro 1988). According to the models of Ribeiro and Spielman (1986) satyrism may be a factor in the parapatric distributions of closely related species or subspecies that live in similar habitats and that have not evolved premating reproductive barriers. The parapatric distributions of several closely related taxa, and the relative ease of obtaining inter-taxon matings in the laboratory, suggest that some tsetse taxa may be candidates for control by satyrs.

Ribeiro (1988) suggested that Vanderplank's success (Vanderplank 1947) in eradicating a population of G. swynnertoni by release of G. m. centralis may have been due as much to asymmetrical satyrism as to hybridization and hybrid male sterility. In the simplest case mentioned above, the satyrs simply function as sterile males but in Vanderplank's experiment there were the additional complications that F_1 males and probably some of the backcross males were sterile while the introduced males and some of the backcross males were not.

The introduction of satyrs, into the territory of a species with which they will mate, has several potentially attractive features as a genetic control method. Only one colony of insects, the source of the satyrs, needs to be maintained (an advantage over release of F_1 hybrid males), and the males that are being released will not have been subjected to either radiation or chemicals, with the possible concommittant somatic damage. Maintenance of

a single colony and the lack of need for radiation equipment offers further economic advantages to the satyr release method.

There are however, some unresolved potential problems with the proposed use of satyrs. The satyrs must mate with females of the target species, in nature, as effectively as do the males of the target species, and females of the target species that mate with satyrs must behave as though they had mated with their own species. Since it is likely that a reasonably high proportion of female tsetse flies in natural populations mate more than once, it is important that these females use the satyr's sperm as often as they use the sperm of the males from their own species. If they do so, the fecundity of twice-mated females will be reduced. However if females are able to preferentially use the sperm of males from their own taxon, the satyrs will have a less significant effect on the fecundity of the twice-mated females. Before evaluating the potential of satyrs one must know whether twice-mated female tsetse flies use sperm from both matings, and if they can distinguish between sperm of their own taxon and that of a satyr. Below I describe experiments undertaken to address the questions of sperm use by twice-mated female tsetse flies.

Use of sperm by twice-mated G. m. morsitans

Mating, receptivity and the question of multiple matings by female tsetse flies have been reviewed by Tobe and Langley (1978) and Gooding (1984a). For most species there is no doubt that, although receptivity of females declines after mating, some females will mate more that once. For example, under laboratory conditions, about 40% of G. pallidipes females will remate during the first two weeks of adult life if given the opportunity to mate each day (Jaenson 1979). Under field conditions about 12% of the females of this species taken in copula at bait animals were already inseminated (Rogers 1973). That females may use sperm received during two matings has been demonstrated in G. austeni by mating them with fertile and sterilized males (Curtis 1968b, 1968c, 1970a) and in G. m. morsitans by matings with fertile and sterilized males (Dame and Ford 1967) or with wild type and genetically marked males (Kawooya 1977, Van der Vloedt 1980). However, the questions of sperm precedence (i.e. the influence of the order of insemination upon the order of sperm utilization) and of capacitation of sperm (i.e. post-insemination enhancement of the capacity of sperm to fertilize eggs) have not been considered in tsetse flies. Below I present evidence on these two questions using genetically marked G. m. morsitans.

In the first experiment I used G. m. morsitans from two inbred lines that had different alleles for the autosomal gene coding for octanol dehydrogenase (Odh). Twenty-six females that were from a line homozygous for Odh^1 were mated individually to males from the same inbred line and then to males from a line homozygous for Odh^f. Males were electrophoresed to confirm their genotypes using techniques described by (Gooding and Rolseth 1979, 1982).

Females were maintained isolated in small cages and each was electrophoresed, to confirm her genotype, after she had deposited three or four larvae. Adults emerging from puparia were maintained in isolated, labelled cages for several days and then electrophoresed. The results (Table 3) indicated that 58% of the females used sperm from the first mating only, 38% used sperm from both matings, and 4% used sperm from the second mating only. For the 16 females that used sperm from one mating only, there was no assurance that they had received sperm during the other mating.

Data for 10 females that used sperm from both matings indicated that females preferentially used sperm from the first mating for the first two pregnancies and then preferentially used sperm from the second mating for the

Table 3. Use of sperm by twice-mated G. m. morsitans.[1]

Number of offspring	Number of females using sperm from First male only	First and second males	Second male only
3	6	3	1
4	9	7	0

[1]Females and males used first were Odhi/Odhi, males used second were Odhf/Odhf.

Table 4. Order of sperm utilization by twice-mated G. m. morsitans[1].

Number of females	Odh Genotypes of Offspring Number 1	2	3	4
4	i/i	i/i	i/i	i/f
2	i/i	i/i	i/f	i/f
1	i/i	i/f	i/f	i/f
2	i/i	i/i	i/f	-2
1	i/i	i/f	i/f	-2
[3]Number from sperm of				
First mating	10	8	4	0
Second mating	0	2	6	7

[1]Females are those referred to in Table 3 that used sperm from both matings.

[2]These females produced only three offspring each.

[3] $\chi^2 = 20.41$, 3 d.f. $p < 0.001$.

third and fourth pregnancies (Table 4). The reasons for this are unknown and it is difficult to envisage a physical sorting of the sperm in the spermathecae that would give an advantage to the Odh^i type sperm in early pregnancies and to Odh^f type sperm in the later pregnancies. One possible explanation is that the marker Odh^f is associated with a factor that slowly kills or incapacitates the sperm from the line carrying the marker Odh^i. It is also possible that there is capacitation of the sperm, although this phenomenon has not been demonstrated in insects. The results could be explained if the sperm of type Odh^f are capacitated much more slowly than those of type Odh^i, at least in females of the Odh^i/Odh^i inbred line.

Thus, at the time of the first fertilizations the sperm of type $\underline{Odh}^{i}$ had a competitive advantage. However, as sperm of type $\underline{Odh}^{i}$ were selectively depleted in the first pregnancies, and as the sperm of type $\underline{Odh}^{f}$ were capacitated, the probability of using sperm of type $\underline{Odh}^{f}$ increased in the later pregnancies. A third possibility is that sperm from both matings are temporarily mixed together and then sperm from $\underline{Odh}^{i}/\underline{Odh}^{i}$ males move to the outside of the sperm mass because of a greater affinity (mediated by something similar to a histocompatibility factor) for the spermathecal wall of $\underline{Odh}^{i}/\underline{Odh}^{i}$ females. If such a mechanism existed, it probably has nothing to do with the Odh locus but rather another locus that is fortuitously different in the two inbred lines. Obviously there is a need to confirm these results with larger samples in experiments using reciprocal crosses of several inbred lines.

A test of Eberhard's sexual selection hypothesis

The above data indicate that use of sperm by twice-mated females may be more complex than would be indicated by the apparently simple structure of the sperm storage and transfer system of tsetse flies. Eberhard (1985) has suggested that females may be influenced to use sperm by stimuli received during mating and that males may vary in their ability to stimulate females. Simply put, females that receive the appropriate stimuli use the sperm received and those that fail to receive such stimuli do not. For example, male G. m. submorsitans transfer sperm to G. m. morsitans but the females do not use the sperm (Gooding 1985, 1989), possibly because the females did not receive appropriate stimuli. This suggestion is an alternative to the proposal that fertilization did not occur due to maternally inherited sterility factors (Gooding 1987, 1989) as an explanation of the asymmetry in success in hybridizing subspecies of G. morsitans.

To test the above suggestion, virgin females from inbred, genetically marked lines were mated twice, once with a male from the same line and once with a male from a genetically marked, inbred line of a different subspecies. Females that mated twice were each retained in an isolation cage and the puparia collected, adults allowed to emerge and these were electrophoresed when about one week old. Each subspecies of G. morsitans carried different alleles for the autosomal marker gene Mdh (Gooding 1982b), and the subspecies of G. palpalis carried different alleles for the autosomal marker gene Est-1 (Gooding 1982b). In most experiments reciprocal crosses were made and experiments were done to test the effect of the order in which females were offered consubspecifics or satyrs.

When using females of G. m. morsitans, G. m. submorsitans, G. p. palpalis, or G. p. gambiensis, the order in which mating opportunities occurred had no significant effect upon whether the females would mate a second time (Table 5). However G. m. centralis females were far less likely to remate after they they had mated with G. m. centralis males than after they had mated with G. m. submorsitans males (Table 5).

There are three patterns of sperm use by females that mated with males of their own subspecies and with satyrs. The 57 females of the three subspecies of G. morsitans, with a single exception, used only the sperm of their own subspecies, and not that of the satyr. In contrast, G. p. palpalis females used sperm from males of their own subspecies, the satyr or both. However, to date, females of G. p. gambiensis used either sperm of their own subspecies or both but never that of the satyr alone.

Table 5. Mating female tsetse flies with consubspecifics and with satyrs.1,2

	Taxon of males		Number of Females	
Female Taxon	First Mate	Second Mate	Mating Once	Mating Twice
1) G. m. c.	G. m. s.	G. m. c.	5	20
2) G. m. c.	G. m. c.	G. m. s.	25	2
3) G. m. s.	G. m. c.	G. m. s.	11	13
4) G. m. s.	G. m. s.	G. m. c.	12	13
5) G. m. m.	G. m. m.	G. m. c.	13	10
6) G. m. m.	G. m. c.	G. m. m.	16	9
7) G. p. p.	G. p. p.	G. p. g.	2	11
8) G. p. p.	G. p. g.	G. p. p.	3	7
9) G. p. g.	G. p. p.	G. p. g.	8	3
10) G. p. g.	G. p. g.	G. p. p.	6	6

1 Taxa abbreviations: G. m. c. = G. m. centralis; G. m. m. = G. m. morsitans; G. m. s. = G. m. submorsitans; G. p. g. = G. p. gambiensis; G. p. p. = G. p. palpalis.

2 $\chi 2$ (with Yates correction) comparisons of the lines in the table (each with 3 d.f.): lines 1 and 2, $\chi 2 = 25.13$ ($p < 0.001$); lines 3 and 4, $\chi 2 = 0.02$ (n.s.); lines 5 and 6, $\chi 2 = 0.05$ (n.s.); lines 7 and 8, $\chi 2 = 0.11$ (n.s.); lines 9 and 10, $\chi 2 = 0.47$ (n.s.).

If Eberhard's hypothesis had applied to the models studied here, one would have expected that G. m. centralis and G. m. submorsitans females that mated with both G. m. centralis and G. m. submorsitans would have been sufficiently stimulated by males of their own subspecies to use whatever sperm were in the spermathecae, and a certain number of offspring sired by satyrs would occur. This did not happen. The results of the experiment in which G. m. morsitans females were used was unexpected. G. m. centralis males are about as effective as G. m. morsitans males at fertilizing G. m. morsitans females (Gooding 1985) and yet none of the 14 twice-mated females used G. m. centralis sperm to produce offspring. Overall these results provide no support for Eberhard's hypothesis and they raise doubts about whether satrys would be effective as genetic control agents against the three subspecies of G. morsitans.

To be effective as genetic control agents, satyrs must 1) be competitive, in nature, with males of the target species, in mating with females of the target species, 2) be as effective in inducing monogamy, and 3) have sperm that are fully competitive with those of the target species males, if females of the target species mate more than once. For tsetse, there are few data on these points. Jackson (1945) found that G. swynnertoni and G. m. centralis mated non-assortatively in G. swynnertoni habitat. The limited data presented here (Table 5) indicate that, in four of five models, satyrs were as good as males of the target species at inducing monogamy. The exception was that G. m. centralis females were more likely to remate after mating with G. m. submorsitans than after mating with G. m. centralis. Within the G. morsitans subspecies, the spermatozoa of satyrs were much less competitive than were those of consubspecific males; only one of 57 twice-mated females used sperm of a satyr (Table 6). To date, laboratory

Table 6. Sperm use by females mated with consubspecifics and with satyrs.[1]

Female Taxon	Taxon of males: First Mate	Taxon of males: Second Mate	Number of Females[2] using sperm from: Own Taxon	Both Taxa	Satyr Only	Avg. No. Pup.
G. m. c.	G. m. s.	G. m. c.	18	0	0	3.5
G. m. c.	G. m. c.	G. m. s.	2	0	0	3.0
G. m. s.	G. m. c.	G. m. s.	12	0	0	5.0
G. m. s.	G. m. s.	G. m. c.	11	13	0	7.3
G. m. m.	G. m. m.	G. m. c.	7	0	0	6.9
G. m. m.	G. m. c.	G. m. m.	7	0	0	6.0
G. p. p.	G. p. p.	G. p. g.	2	2	3	4.3
G. p. p.	G. p. g.	G. p. p.	2	14	1	6.2
G. p. g.	G. p. p.	G. p. g.	2	1	0	4.5
G. p. g.	G. p. g.	G. p. p.	3	35	0	6.5

[1]Taxonomic abbreviations as listed in Table 5.

[2]Data are for females from which three or more offspring were analyzed.

[3]One offspring sired by G. m. centralis, three by G. m. submorsitans.

[4]Two sired by satyr, five by consubspecific male.

[5]Each female used sperm of satyr for one offspring and sperm of consubspecific for 5, 7, or 8 offspring.

experiments indicate that the best prospect for using satyrs as control agents would be the release of G. p. gambiensis males into a population of G. p. palpalis. In this model the G. p. gambiensis sperm appear to be competitive with G. p. palpalis sperm in twice-mated females, and in a significant proportion (4 of 11) of the double-mated females sperm from the satyrs may have displaced or completely out competed that of the consubspecific males.

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TSETSE FLY MASS REARING: COLONY MANAGEMENT, DEPLOYMENT OF STERILE FLIES, RELATED RESEARCH AND DEVELOPMENT

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Abstract

In support of tsetse fly research and control programmes in Africa, seven economically important tsetse fly species (Glossina palpalis palpalis, G. tachinoides, G. fuscipes fuscipes, G. austeni, G. brevipalpis, G. pallidipes and G. morsitans submorsitans) are maintained at the Entomology Unit of the IAEA Agriculture Laboratory at Seibersdorf, Austria. All flies are reared without host animals and are fed exclusively by the membrane feeding technique. The strains of the first five species above are adapted to mass-rearing procedures and are maintained in numbers sufficient to meet the needs for field releases and for laboratory experiments. The colony of G. tachinoides is maintained at 100,000 females to demonstrate (i) the time required for transition of an adapted strain to a mass-reared colony, (ii) the ability to routinely ship pupae (35,000 every three weeks) a long distance and (iii) the high quality of sterile males originating from transported pupae.

Topics requiring R&D for large scale use of SIT to combat tsetse flies include simplification and automation of the laborious day-to-day colony routine work. Emphasis is placed on procedures that offer potential for sex differentiation in pre-adult stages or that offer a better synchronization of adult emergence with minimal overlapping of the sexes. Development of methods for extending the duration of the pupal period would increase flexibility in preparing for long distance transport of pupae to release projects. For all tsetse fly species colonized at Seibersdorf, radiation dose-response data are

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collected and the quality of the treated fly material evaluated. The receptivity of irradiated virgin females to mating is studied, in view of using them as indicator or sentinel insects for detection and monitoring of native male populations existing at low density or being subjected to control measures.

Introduction

The Sterile Insect Technique (SIT), involving the release of sterile insects of the indigenous target species only, is an environmentally acceptable method of insect control because it is non-polluting, species-specific and does not involve the introduction of other biocontrol agents such as predators, parasites or pathogens with uncertain host selectivity. Its feasibility for use against tsetse fly populations has been demonstrated in several projects in Africa (Williamson et al., 1983; Cuisance et al., 1986; Takken et al., 1986). There has been concern that two characteristics of tsetse flies, i.e. their obligatory hematophagy and their larviparity, may hamper effective mass-rearing. The development of the membrane feeding technique (Bauer and Wetzel, 1976), which eliminated the need of a host animal colony, has been a major breakthrough for a more economic maintenance of tsetse fly colonies. For example, at the BICOT project in Nigeria, in vivo feeding of 60,000 G. p. palpalis colony females involved the maintenance of 2,000 guinea pigs, of which up to 45% could be used as "feeders" (Oladunmade et al., 1990). In vitro feeding of a colony of the same size requires basic feeding equipment and only 25 litres of quality-tested, clean blood (i.e. microbially decontaminated with gamma radiation) per week, which may be collected well in advance and stored frozen until use. Another criticism questions the high cost for mass-production, transport and release of sterile males (Brandl, 1988).

Priorities for work in the Entomology Unit of the IAEA Agriculture Laboratory at Seibersdorf, Austria, in support of SIT are, therefore, (i) to adapt economically important Glossina spp. to mass-rearing conditions, (ii) to economize the mass-production of sterile males, (iii) to develop suitable long distance transport procedures for insects from a centralized breeding center to peripheral release projects and (iv) to further develop the potential of sterile insects for monitoring the progress of ongoing control operations.

Mass-rearing of Glossina spp.

Seven tsetse fly species were maintained at the Entomology Unit during 1990: Glossina palpalis palpalis, G. tachinoides, G. fuscipes fuscipes, G. austeni, G. brevipalpis, G. morsitans submorsitans and two strains of G. pallidipes. Table I summarizes the origin of the strains, the performance of the colonies for the period January to December 1990 and the present status. All flies are fed exclusively by the artificial membrane feeding technique. G. pallidipes is under adaptation to mass-rearing procedures, which may require two years, and G. m. submorsitans is an experimental colony. The strains of all other species are fully adapted to basic mass-rearing procedures and produce numbers sufficient to meet the needs for release projects and for in-house experiments or requests by collaborating researchers. During 1990, more than 2.32 million pupae were produced. The performance of the colonies was measured by conducting routine quality control measures. Emergence from pupae ranged form 89.8±3.3% (G. p. palpalis) to 94.9±2.6% (G. austeni), daily mortalities ranged from 0.94±0.15% (G. p. palpalis) to 0.32±0.11% (G. brevipalpis) and fecundity of colony females was between 0.76±0.11 (G. f. fuscipes) and 0.94±0.04 (G. austeni) pupae per female per 10 days.

Table I: Performance and status of Glossina spp. colonies at the Entomology Unit, FAO/IAEA Lab, Seibersdorf, in 1990

species	country of origin	% EM rate (mean ± std)	% daily mortality (mean ± std)	No. pupae/ fem./10 d (mean ± std)	No. pupae produced Jan. to Dec. 1990	Mean No. colony females Dec.'90	status of colony
G. tachinoides	Burkina-Faso	91.6 2.8	0.57 0.20	0.87 0.09	1,831,379	109,597	production colony to provide 50,000 excess pupae per month to BICOT, Nigeria
G. p. palpalis	Nigeria	89.8 3.3	0.94 0.15	0.89 0.07	279,960	15,125	stock colony to provide 6,000 pupae pupae per month for experiments
G. f. fuscipes	Central African Republic	90.9 3.4	0.45 0.22	0.76 0.11	51,963	1,225	maintenance stock colonies, adapted to mass-rearing conditions
G. brevipalpis	Kenya	90.3 5.8	0.32 0.11	0.83 0.08	50,441	1,213	
G. austeni	Zanzibar, Tanzania	94.9 2.6	0.64 0.23	0.94 0.04	67,006	1,187	
G. pallidipes	Uganda	84.8 4.5	0.85 0.26	0.84 0.09	31,350	1,080	colonies under adaptation to mass-rearing conditions
	Zimbabwe		0.75 0.33	0.71 0.12	2,897	354	
G. m. submorsitans	Burkina-Faso	94.6 2.4	0.97 0.24	0.52 0.07	6,064	965	experimental colony
					2,321,060		

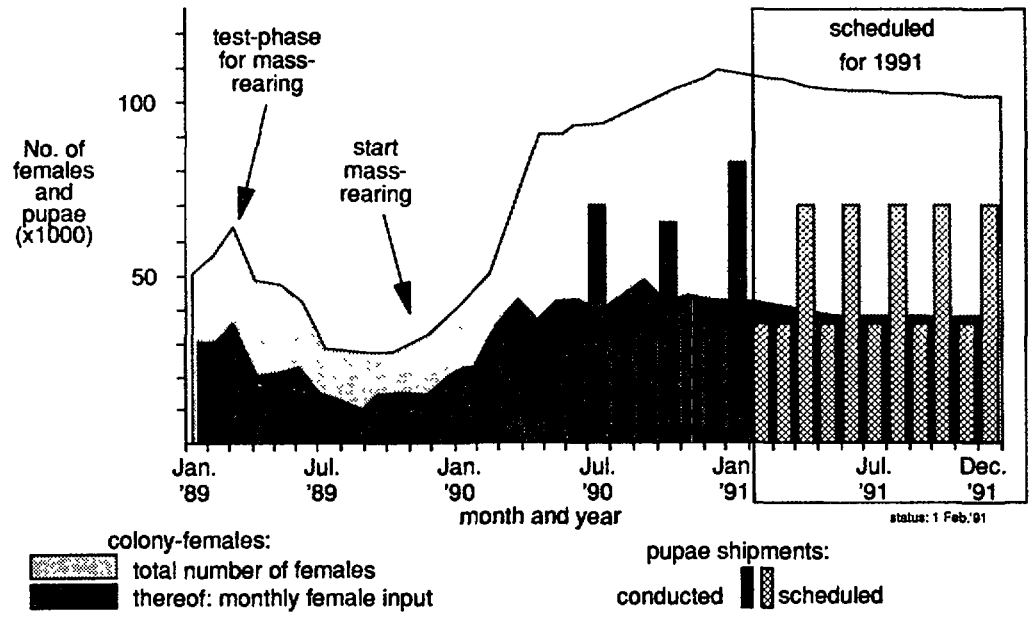


Figure 1: Mass-rearing of *G. tachinoides* at the Entomology Unit, IAEA/FAO Agriculture Laboratory Seibersdorf: Colony development and long-distance pupae shipments for the application of the SIT in Nigeria (1989 - 1991)

Figure 1 shows the development of the G. tachinoides colony as of January 1989. Late in 1988 until March 1989, the colony was allowed to expand but thereafter reduced to below 30,000 females because of low needs for pupae. In November 1989, the decision was made that the Seibersdorf colony was to provide, as of July 1990, 50,000 G. tachinoides pupae per month in support of the BICOT project in Nigeria. Additional priorities were to determine (i) the time required to transfer the adapted strain to a production colony with maximal output, (ii) the ability to routinely ship large numbers of pupae long distances on a time schedule and (iii) the quality of sterile males emerging from transported pupae. The colony was transferred to mass-rearing status with maximum female input and by May 1990 had reached the desired production level. Figure 2 shows the breeding room with arrangements for in vitro feeding of the colony. Female colony units are formed on a weekly basis and are kept in the colony for 13 weeks. Once the G. tachinoides colony had reached the desired size and target pupae production, the weekly input of young females was regulated at approximately 10% of the actual colony size. Subsequent to the separation of sexes after mating, 80 - 100 females are transferred to "MC-20" PVC cages (diameter 20 cm, height 4.5 cm). Each trolley (in Figure 2) initially holds 9,800 females. Pupae (6,000 - 9,000 per day) are collected every morning, except Sundays. Because of low mortality among young colony flies, dead females are first removed from the cages after five weeks and thereafter in fortnight intervals. Flies are offered a bloodmeal five days per week (no feeding on Wednesdays and Sundays). Approximately 30 l of diet are needed per week, consisting of 75% fresh-frozen cattle-, 25% fresh-frozen pigs-blood and 10^{-3} M ATP. The blood is collected at the local abattoir in batches of 150 - 250 l; during 1990, more than 4,500 l were collected.

Figure 2: Mass-rearing of *Glossina tachinoides:* breeding room and *in vitro* feeding of the colony

Pupae Shipments and Releases of Sterile Males

In March 1990, test shipments of 3,000 G. tachinoides pupae were made to establish a routine procedure for long distance shipments from Seibersdorf via airports Schwechat/Vienna, Heathrow, Gatwick and Kano to Vom, Nigeria. The BICOT staff established a procedure of pre-arrival clearance of the shipments with the Nigerian customs authorities, resulting in immediate release of the pupae upon arrival and immediate tranport to the Vom facility. Beginning early July 1990, large numbers of G. tachinoides pupae were shipped at three week intervals. Figure 3 shows a batch of pupae before despatch from the Seibersdorf laboratory. The total transport time was between 35 and 60 hours. Emergence rates from such pupae ranged between 76.8% and 91.2% and thus providing up to 13,000 males for field releases. However, reliable transit connections remain the prerequisite for efficient long distance transport of the pupae Delays have been experienced which have adversely affected the quality of the flies. Releases of sterile G. tachinoides males in Nigeria started at the onset of the dry season 1990/91. The males were handled as described by Oladunmade et al. (1990). The average pre-release losses (i.e. mortality and inability to fly, resulting from handling in the laboratory and transport to the field) were less than 5%.

With current methods, a colony of 100,000 female flies can provide up to 10,000 sterile males per week. The infested area that can be covered with this number of sterile males varies with the habitat, the species concerned and the level of pre-release population suppression. For example, in the Southern Guinea zone of Nigeria, eradication of G. p. palpalis from dense

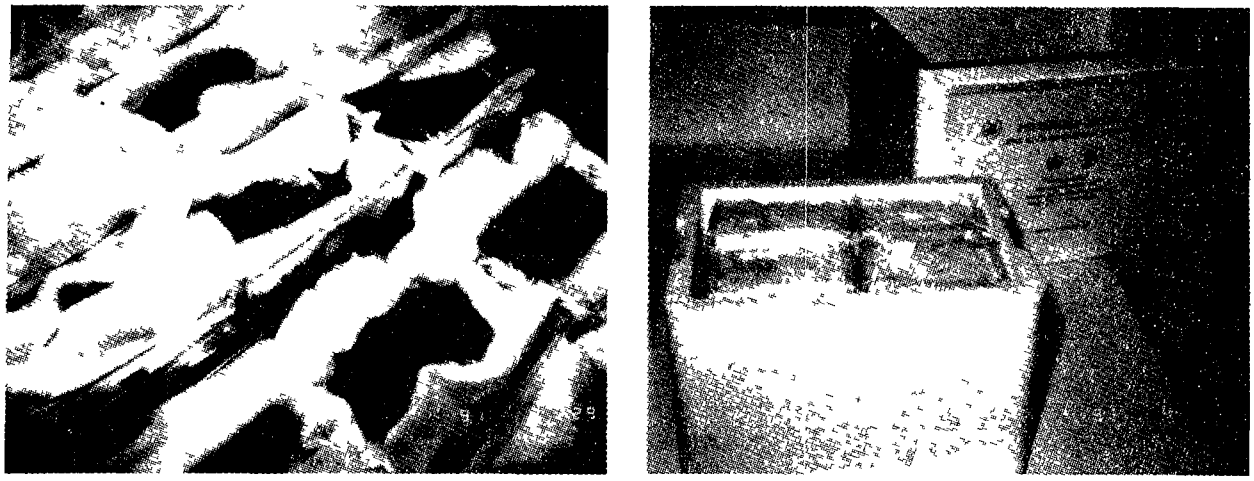


Figure 3: 35,000 *Glossina tachinoides* pupae before despatch from Entomology Unit at Seibersdorf

riverine forests was achieved by a combination of traps and impregnated targets for three months followed by weekly releases of 90 sterile males per linear km for a period of at least 8 months.

R&D for Glossina spp. Mass-rearing

Some aspects of the day-to-day mass-rearing work are very laborious. For example, with the Seibersdorf G. tachinoides colony, 20% of the insectary staff's weekly working hours are needed to feed the colony (five feeding days per week). A preliminary test was conducted to explore the feasibility of an automatic in vitro feeding system (hereafter referred to as AFS). Ideally, AFS should reduce the time required for feeding flies, should be applicable for various in vitro reared Glossina spp. and should be independent from the type of cage used for the species. The system that has been developed consists of a mobile frame, the size of a feeding membrane, which can hold different types of cages by means of a grid on one side to support the cages but which will still permit larvae to fall through, and with a removable net on the other side. During feeding, the flies project their mouthparts through a double layer of netting before piercing the membrane. Figure 4 shows a prototype of this cage-holding and feeding frame. Four MC-20 cages, each with 100 mated G. tachinoides females, were placed in the holding frame and their performance was recorded for 12 weeks. As control, an equal group of females was held in MC-20 cages. From the data in Figure 5, it is obvious that the system of a holding frame and a removable top does not harm the flies and may be used as a basic unit for further development of an AFS.

About 40% of the available man hours are spent manually separating males and females after emergence and after mating with short chilling at +4°C. Sex recognition at an earlier stage would not only reduce labour but also increase efficiency by immediate selection of male pupae earmarked for shipment to SIT projects. Table II shows the duration of both the free third instar larva stage and pupal stage, during which sex-separation could be done. Duration of the free third instar larva stage differs among Glossina species from 7 minutes for G. austeni larvae to 100 minutes for G. tachinoides, but no significant sex-related differences were found among the species (Table III). Subsequent work on third instar larvae was focussed on G. tachinoides which has a relatively long crawling time. The diurnal larviposition pattern of the G. tachinoides colony (Figure 6) shows that almost two thirds of the larvae are deposited between 12.00 and 04.00 hours.

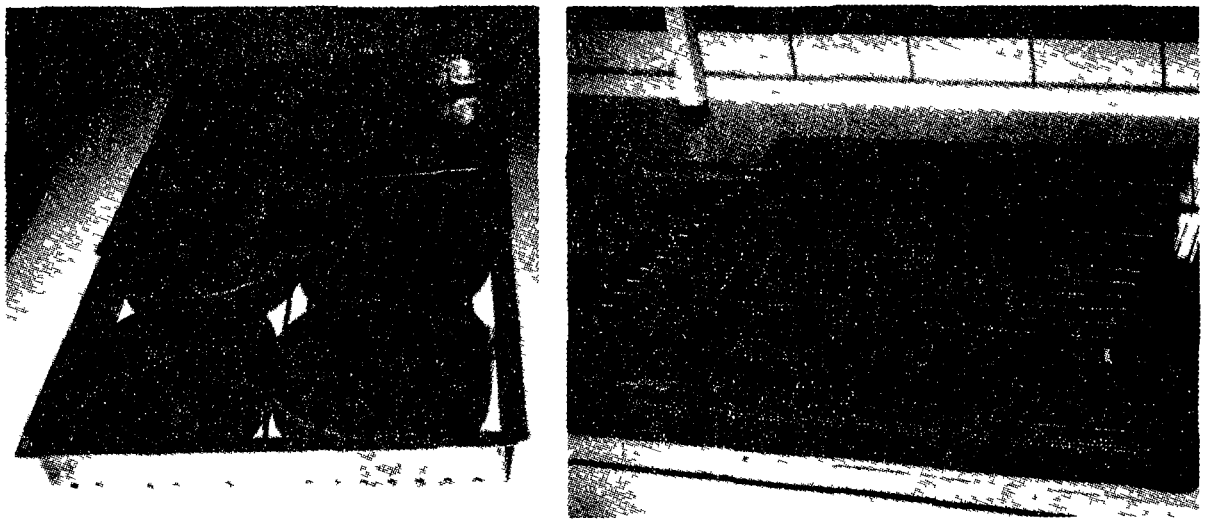


Figure 4: Cage-holding and feeding frame as a basic unit for an Automatic Feeding System (AFS) for *Glossina* spp.
left: holding position, top-net is removable; right. feeding position

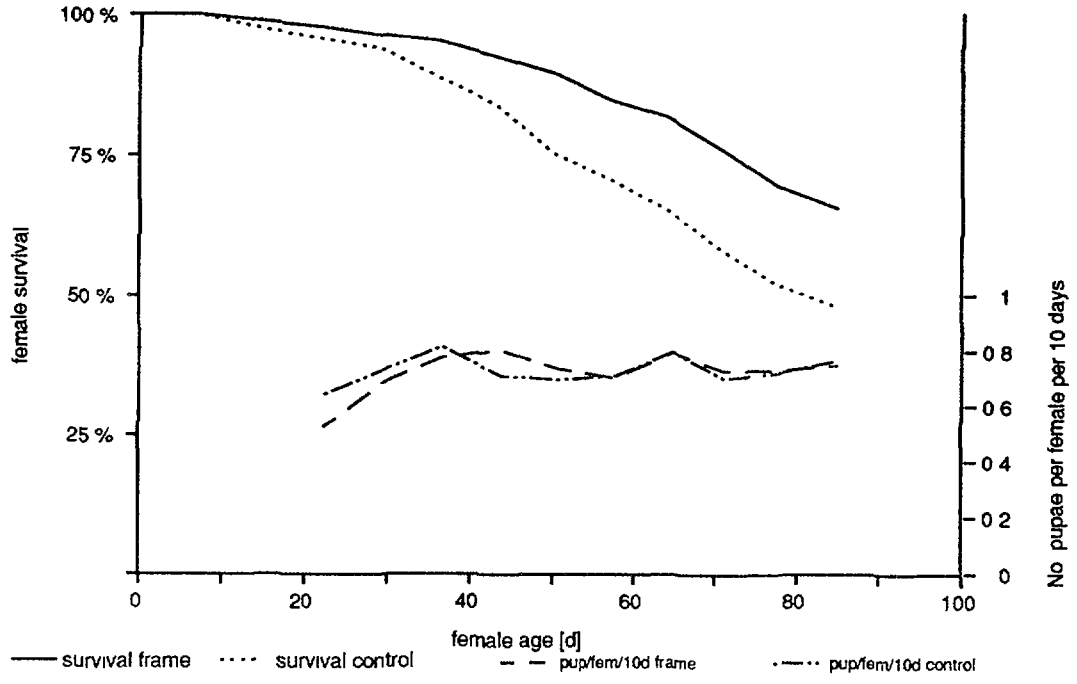


Figure 5: Survival and fecundity of *Glossina tachinoides* females; "feeding-frame" vs. "normal" *in vitro* feeding

Table II: Duration of immature tsetse fly stages and applicability of a sex separation method

stages	free third instar larva	pupae stage early	pupae stage mid	pupae stage late	emergence
duration	G. austeni 7 min G.p.palpalis 1 h G. brevipalpis 1 30 h G. tachinoides 1 40 h		30 to 40 days		5-8 days
application	for insect material before pupae shipment and for retained material				for retained material only

Table III: Duration of the free third instar larva stage of *Glossina* spp.

species	larvae->males	larvae->females	significant difference
G. tachinoides	105 ± 17 min	110 ± 36 min	no
G. brevipalpis	97 ± 29 min	99 ± 27 min	no
G. austeni	9 ± 7 min	5 ± 4 min	no
G. p. palpalis	73 ± 29 min	44 ± 21 min	no

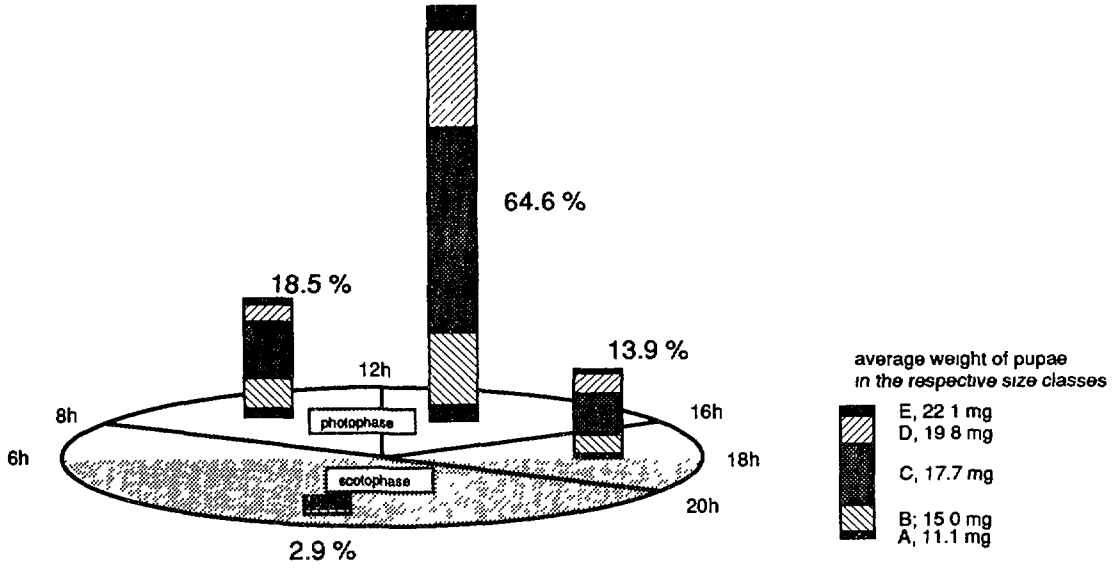


Figure 6: Diurnal larviposition pattern in the *Glossina tachinoides* colony:
Larvae and pupae (in total 3,994) were collected four times per day for 48 hrs. and grouped into size classes. Almost two thirds of the daily production was was recorded between 12 h and 16 h, less than 3 % between 20 h and 8 h.

This peak in larviposition and the long free larva stage provide the opportunity to apply test treatments to considerable amounts of fly material at the same developmental stage. At first, freshly deposited larvae were transferred to a physiological saline solution for 0.5, 1, 3, 6 and 12 hours. After removal from the solution, the time to pupation was recorded. Figure 7 shows that treating larvae for one and three hours resulted in heavier initial pupae weight. Longer saline treatments, particularly for 12 hours, led to more pronounced weight losses and low emergence rates. Generally, a one to three hour saline treatment of male larvae resulted in a larger reduction of the average crawling time than for female larvae. The difference was significant in the group treated three hours (Table IV).

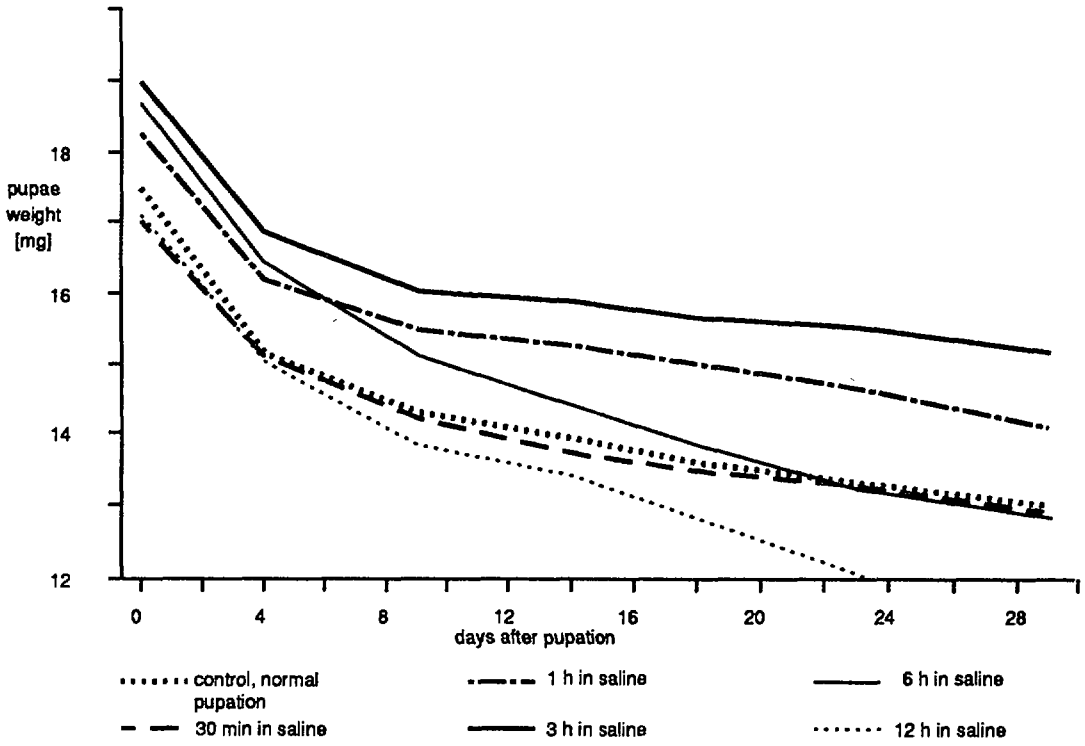


Figure 7: Weight loss of *Glossina tachinoides* pupae, kept as third instar larvae for different times in saline

Table IV: Effect of saline treatment on the duration of the free third instar larva of *Glossina tachinoides*

treatment	larvae->males	larvae->females	significant difference
"normal" pupation	105 ± 17 min	110 ± 36 min	no
3rd instar larvae 2 h in saline	29 ± 22 min	51 ± 33 min	yes (P=5%)

A few other findings were made in the search for morphological or physiological differences among immature stages of male and female Glossina spp. For example, between day 6 and day 12 after larviposition, G. p. palpalis male pupae lost 0.2 - 0.3 mg more weight than female pupae (Figure 8). Among G. tachinoides, a morphological difference was found regarding the ratio of length to width of male and female pupae. Table V shows that the ratio for male pupae is in most replicates significantly higher, despite the small sample sizes and different treatment of the larvae. Figure 9 shows that with this method, 90% of the males may be identified within a few days after larviposition (however, with 37% female contamination), and a portion of females may be selected that has about 11% male contamination. In addition, procedures are sought for treatment and incubation of pupae that might enhance a better synchronization of adult emergence with minimal overlapping of the sexes. Although the differences detected among immature male and female stages do not permit an absolute sex identification, a combination of them and/or automation of different methods could substantially contribute to a more efficient system of tsetse fly mass-rearing.

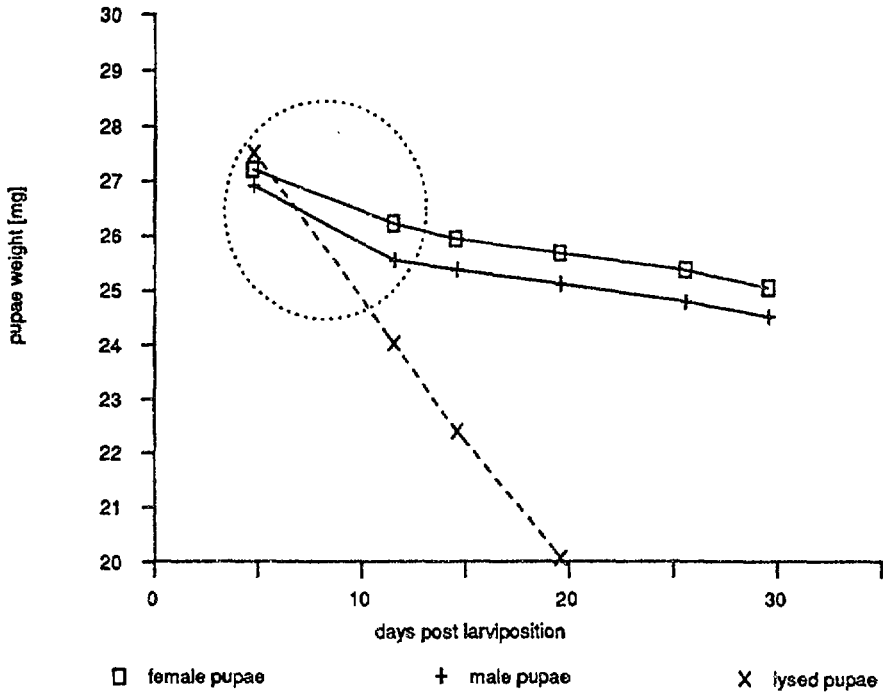


Figure 8: Weight loss of male and female *G. p. palpalis* pupae

Table V: Ratio of length to width of *G. tachinoides* pupae three days after larviposition

treatment of 3rd instar larva	ratio length : width of male puparia (n, avg stdev.)	significant difference	ratio length : width of female puparia (n, avg stdev.)
normal pupation	21, 1.96 0.08	yes (P=1%)	18, 1.88 0.06
3 hrs. saline	19, 1.96 0.08	yes (P=1%)	15, 1.87 0.08
normal pupation	6, 1.98 0.03	yes (P=0.1%)	22, 1.88 0.07
3 hrs. water	8, 1.94 0.04	no	12, 1.92 0.07
normal pupation	15, 1.92 0.09	no	29, 1.87 0.12

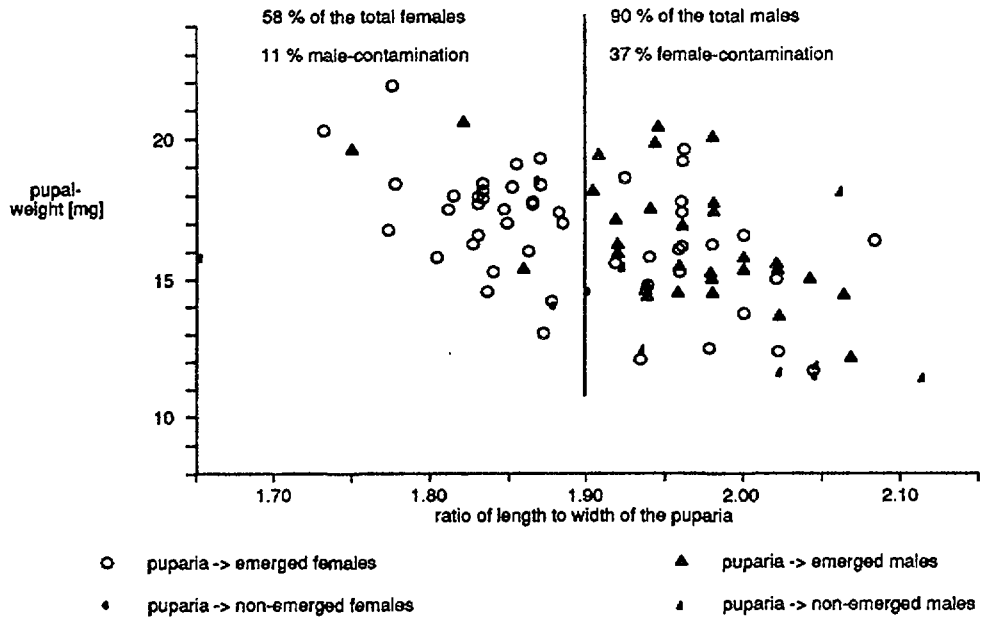


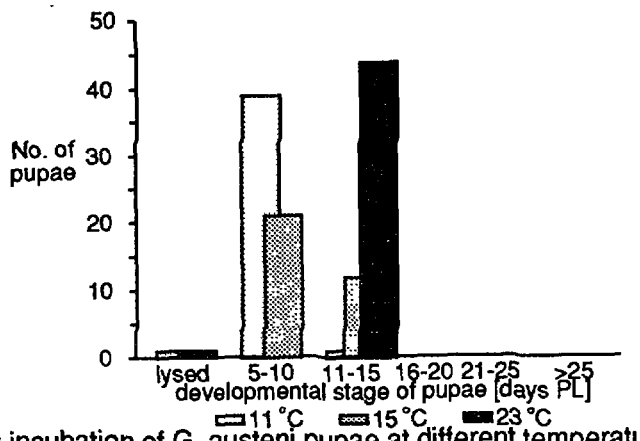
Figure 9: ***Glossina tachinoides:*** **Ratio of length to width of three day old male and female puparia**

Extension of the Pupal Period

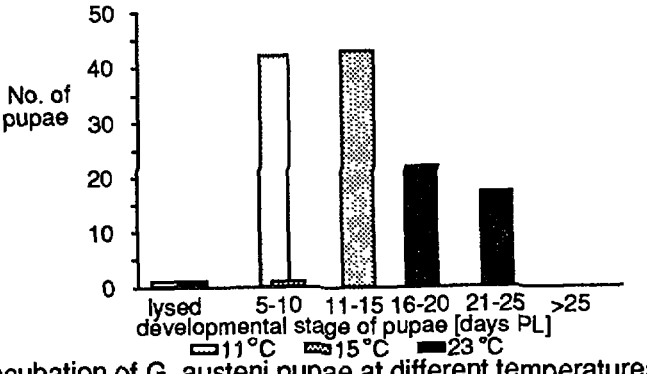
Because of the low reproductive capacity of the tsetse fly, the doubling time for a colony is at least 2 - 2.5 months. Demands for sterile males for operational releases therefore require timely planning of colony outputs. However, unexpected trends in the wild target population may require immediate changes in the production level and deployment rate of flies. This draws attention to the need of extending the shelflife of insects so that they can be used according to real needs.

The effect of ambient temperatures on the duration pupal period in Glossina spp. has previously been studied for various purposes (Buxton, 1955). At Seibersdorf, incubation tests were conducted with changing temperatures and at different developmental stages. Starting at day 4 after larviposition, G. austeni pupae were held at 11°C or 15°C for 9, 15 and 23 days. Corresponding controls were continuously kept at 23°C. One week after temperature treatments, pupae samples from each group were dissected to determine their developmental stage. The number and pattern of emergence from the remaining pupae were recorded, adults from treated pupae were mated in a 3 to 5 male to female ratio with untreated flies of the complementary sex. The survival and pupae production of the females was recorded for 25 days. Thereafter, surviving females were dissected to determine insemination.

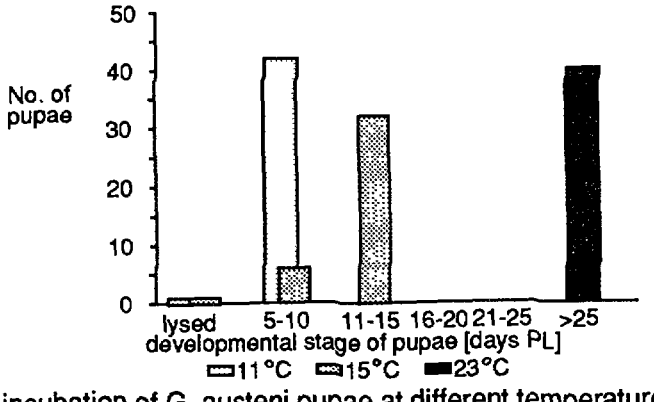
Figure 10 summarizes the developmental stages of pupae one week after the low-temperature treatments. Compared with the 23°C controls, the delay in pupal development among the groups exposed to low temperatures became more pronounced with increase in incubation time. Figure 11 shows the period and rate of adult emergence from different groups of pupae. For pupae maintained continuously at 23°C, an emergence rate of 95.7% was recorded, on average 36.7 days after larviposition. A 9-day incubation at 15°C and 11°C affected a delayed average emergence of 4.1 and 3.9 days respectively, without any apparent influence on the emergence rate (96.7% and 93.3%). The respective treatment groups incubated for 15 days showed an average delay in emergence of 12.6 and 14.7 days. However, the emergence rate was slightly



9 day incubation of G. austeni pupae at different temperatures; developmental stage 15 days after larviposition



15 day incubation of G. austeni pupae at different temperatures; developmental stage 23 days after larviposition



23 day incubation of G. austeni pupae at different temperatures; developmental stage of pupae 30 days after larviposition

Figure 10: State of 15, 23, and 30 day old *G. austeni* pupae after incubation at temperatures of 11, 15, or 23 °C

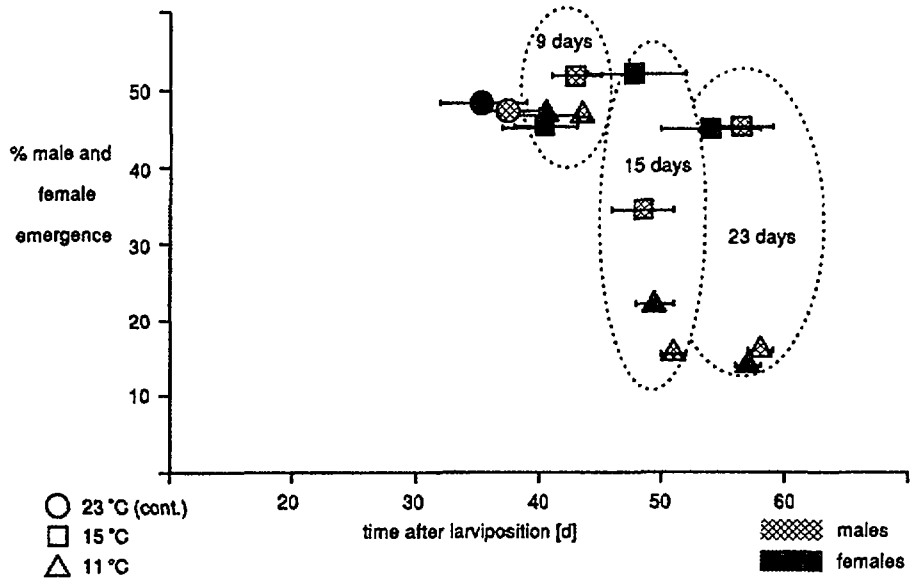


Figure 11: Percent and period of male and female emergence from *G. austeni* pupae incubated for different periods at 11 °C, 15 °C or continuously 23 °C

affected in the group incubated at 15°C (86.7%) but drastically reduced among the group incubated for 15 days at 11°C (37.8%). A greater delay was recorded for the pupae groups incubated for 23 days at 15°C and 11°C, i.e. 18.1 and 21.1 days respectively. The extended incubation at 11°C led to an emergence rate of only 30%, whereas pupae incubated for 23 days at 15°C had an emergence of 90%. Incubation of male pupae at low temperature for 9 days did not have any apparent adverse effect on their ability to inseminate untreated females or on the pupal production of these females. Insemination of untreated females mated by males from the 15 to 23 day low-temperature treatment groups was slightly reduced. However, only females mated by 11°C-group males showed reduced pupal production. Prolonged exposure of female pupae to low temperatures, particularly at 11°C for 15 days or longer, resulted in losses in female receptiveness for mating and reduced fecundity. The results indicate that by incubation at approximately 15°C, the pupal period of G. austeni may be extended to more than 55 days without losses in fly emergence and with only slight reductions in adult performance. However, extended incubation at slightly lower temperatures may result in drastic reduction in emergence and adult performance.

Other Research in Support of SIT

At Seibersdorf, optimum and adverse conditions for a long distance pupae transport were determined for all strains of tsetse fly species which are adapted to basic mass-rearing procedures. Also, radiation dose-response data were collected for males and females of all colonized Glossina species. Finally, efforts at the Entomology Unit at Seibersdorf focus on the possibility to ship sexually sterile female pupae long distances to field projects, releasing virgin females to detect relic fly populations at a density which is below detectable level by conventional trapping (Van der Vloedt and Barnor, 1984).

Acknowledgement

We thank Dr. R.E. Gingrich, Head of the Entomology Unit, IAEA/FAO Agriculture Laboratory, Seibersdorf, for helpful discussions.

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Part V

VECTOR CONTROL: TRAINING, USE OF TARGETS AND COUNTRY PROGRAMMES

A STUDY OF FACTORS AFFECTING THE PERSISTENCE OF DELTAMETHRIN APPLIED TO COTTON FABRIC FOR TSETSE FLY CONTROL

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Abstract

The effect of selected lipophilic materials on the loss of deltamethrin applied to cotton fabric and washed with water was studied. Corn oil, paraffin, linseed oil and silicone oil reduced the leaching of deltamethrin by water from the fabric. The cumulative sum of four washings of the treated cotton strips resulted in a total loss of 37.7% of deltamethrin from the cotton strip (without protectant) and 9.9% from the strip treated with deltamethrin and corn oil. The effect of the colour of fabric and a UV absorber compound on the photodegradation of deltamethrin were also studied. Photodegradation was much less on the blue or black fabric than on the white fabric, and 2,4-dihydroxy-benzophenone reduced the photodegradation of deltamethrin applied to cotton fabric.

Introduction

The use of insecticide-impregnated traps and screens for the control of tsetse flies (Glossina species) in Africa has proved to be very successful (1). Initially, traps were developed to catch tsetse flies (2). More recently, the traps have largely been replaced by cloth screens treated with insecticides (3, 4). The tsetse flies seem to be attracted visually to the screens and prefer certain colours, especially royal blue (5, 6).

Among the synthetic pyrethroid insecticides, deltamethrin has been reported to be most toxic to tsetse flies (7, 8). However, on exposure to sunlight, it degrades rapidly (9). The UV absorber compounds have been found to protect deltamethrin and other pyrethroid insecticides against UV radiation (10). In rainy conditions, the insecticides may be leached away with water. Therefore, under the tropical conditions of rain and intense sunlight, more effective tsetse control screens and traps would require treatment with insecticide formulations which stabilize the insecticides against the effects of sunlight and rain.

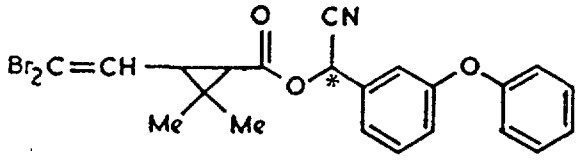
We carried out experiments to study the effect of a number of lipophilic materials on the leaching of deltamethrin with water from cotton fabric. We also studied the effect of the colour of fabric and the effect of a UV absorber compound on the photodegradation of deltamethrin applied to cotton screens.

Materials and Methods

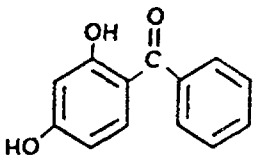
Chemicals

[^{14}C-Benzyl] deltamethrin (Figure 1) specific activity 59mCi mmol^{-1} and radiochemical purity 95% was donated by Roussel Uclaf, Paris, France and Uvinul-400 (2,4-dihydroxy-benzophenone) was supplied by BASF, Limburgerhof, Germany. Paraffin, stearyl alcohol and cetyl alcohol were purchased from

E. Merck, Darmstadt, Germany; corn oil from Sigma Chemical Company, St. Louis, MO, USA; and linseed oil was purchased from Carl Roth KG. Karlsruhe, Germany. Silicone oil (dimethyldiphenylsiloxane) known as Dow Corning 704 fluid was provided by Professor G.G. Allan, University of Washington, Seattle, USA.



Deltamethrin
(S)- α-cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropan-1-carboxylate



Uvinul -400
2,4-dihydroxybenzophenone

* denotes Carbon-14 label

Fig. 1 Chemical structures of deltamethrin and Uvinul-400

Treatment of cotton fabric with [^{14}C] deltamethrin and leaching with water

Strips (1 cm x 16 cm) of white cotton (100%) were treated with 400 ul toluene solution containing 533 ug [^{14}C] deltamethrin and 25 mg of lipophilic material. Prior to treatment, the cotton fabric had been rinsed in deionized water and air-dried. The lipophilic materials used in combination with deltamethrin were blockform paraffin (mp 42°C), granular paraffin (mp 52°C), stearyl alcohol, cetyl alcohol, linseed oil, corn oil and silicone oil. Each treatment was replicated three times. The treated strips were allowed to dry at room temperature overnight before these were subjected to leaching with water. Each dried cotton strip was then transferred into 200 ml deionized water in a polyethylene bottle. The bottle was capped and shaken on a laboratory shaker for one hour. The strip was removed from the water and the radioactivity released into water was quantitated. A 4 ml aliquot (x4) from each leachate was mixed with 11 ml Aquasol (R) Universal cocktail (NEN Research Products, Boston, MA, USA) and the radioactivity in the resulting gel was counted in a Model LS 7800 liquid scintillation counter (Beckman, Fullerton, CA, USA). After leaching, the cotton strips were hung to dry at room temperature. The leaching test was repeated at weekly intervals for a total of four times.

Treatment of cotton fabric with deltamethrin and irradiation under UV lamp

Squares of fabric (1.4 cm x 1.4 cm) were treated, each with 30 ul acetone solution containing 66.6 ug deltamethrin and varying amounts of Uvinol-400 (0, 33.3, 66.6 and 133 ug per square piece of fabric) to give 0:1, 0.5:1, 1:1 and 2:1 ratios of Uvinul-400 and deltamethrin (w/w). Each treatment was replicated three times.

The treated samples were allowed to dry at room temperature for one hour and placed on aluminium foil spread on a copper platform. Water from a Model FJ (Haake, Berlin, Germany) controlled-temperature bath circulated through the copper platform to maintain a constant temperature of 35 ± 1°C on the platform surface. The samples were irradiated with an Osram UV-sun radiation lamp Model Ultra-Vitalux Sonnenstrahler (Osram, Vienna, Austria) which emitted

radiation simulating the spectral distribution of natural sunlight. The lamp is designed to provide radiation intensity equivalent to mid-day natural sunlight (1 KW m^{-2} = 1.42 cal cm^{-2} min^{-1}) at the irradiated surface, when the lamp is adjusted 50 cm above the surface. The lamp was adjusted at a distance of 12.5 cm above the samples and provided radiation intensity equivalent to 16 times that of mid-day natural sunlight. The samples were irradiated for 6, 12 and 24 hours to simulate exposure to mid-day sunlight for the equivalent of 96, 192 and 384 hours, respectively.

Extraction and analysis of deltamethrin

The fabric squares were extracted with acetone and analysed. Each treated cotton fabric square was transferred into a glass vial and 2 ml acetone added. The vial was sonicated for 5 minutes and allowed to stand for 30 minutes. Extraction was repeated one more time. The two extractions extracted 98.4% of deltamethrin applied to the fabric. The two extracts were combined and the concentration of deltamethrin in the combined solution was determined with a HPLC (Waters Associates, Milford, MA, USA) equipped with a Lichrosorb RP-18 column and a Lambda-Max Model 481 LC spectrophotometer detector (Waters Associates, Milford, MA, USA). The eluting solvent was methanol + water (85 + 15 by volume) and the flow rate was adjusted at 1.5 ml min^{-1}. The detection wavelength for the spectrophotometer was set at 225 nm.

Results and Discussion

Protection of deltamethrin from leaching with water

The lipophilic materials used (5% of the weight of the fabric) provided varying degrees of protection to deltamethrin from leaching with water. As shown in Figure 2, when no lipophilic material was added to deltamethrin, 27.10% of the applied insecticide was leached with water during the first leaching test. In contrast, lesser amounts of deltamethrin leached into water when one of the tested lopophilic material was also present on the fabric, indicating that the presence of the lipophilic material would reduce the loss of deltamethrin from the cotton fabric when exposed to water. The degree of protection varied from one protective material to the other, and increased in the order: corn oil > paraffin (mp 52°C) linseed oil > silicone oil > paraffin (mp 42°C) > cetyl alcohol > stearyl alcohol. Corn oil, paraffin (mp 52°C) and linseed oil provided the best protection to deltamethrin from leaching by water, and when the insecticide solution containing one of these materials was applied to cotton fabric, the amount of deltamethrin leached by one washing was 4.2%, 5.1% and 5.6% in the presence of corn oil, paraffin (mp 52°C) and linseed oil, respectively. There was no significant difference among these treatments when data were analyzed by Duncans' multiple range test ($F = 2.66$; $df = 7,16$; P 0.05). As shown in Figure 2, data represented by bars topped by the same letter are not significantly different at 0.05 level. Data shown in Table 1 show that subsequent washing of each strip resulted in additional leaching of deltamethrin. However, the additional washings resulted in leaching of smaller quantities of deltamethrin. Cumulative sum of four washings of the treated cotton strips resulted in a total loss of 37.7% of deltamethrin from the control strip (without protectant) and 9.9% from the strip treated with deltamethrin and corn oil. Based on this information, it can be concluded that corn oil, paraffin (mp 52°C), linseed oil and silicone oil can substantially reduce the loss of deltamethrin from cotton screens exposed to washing with water.

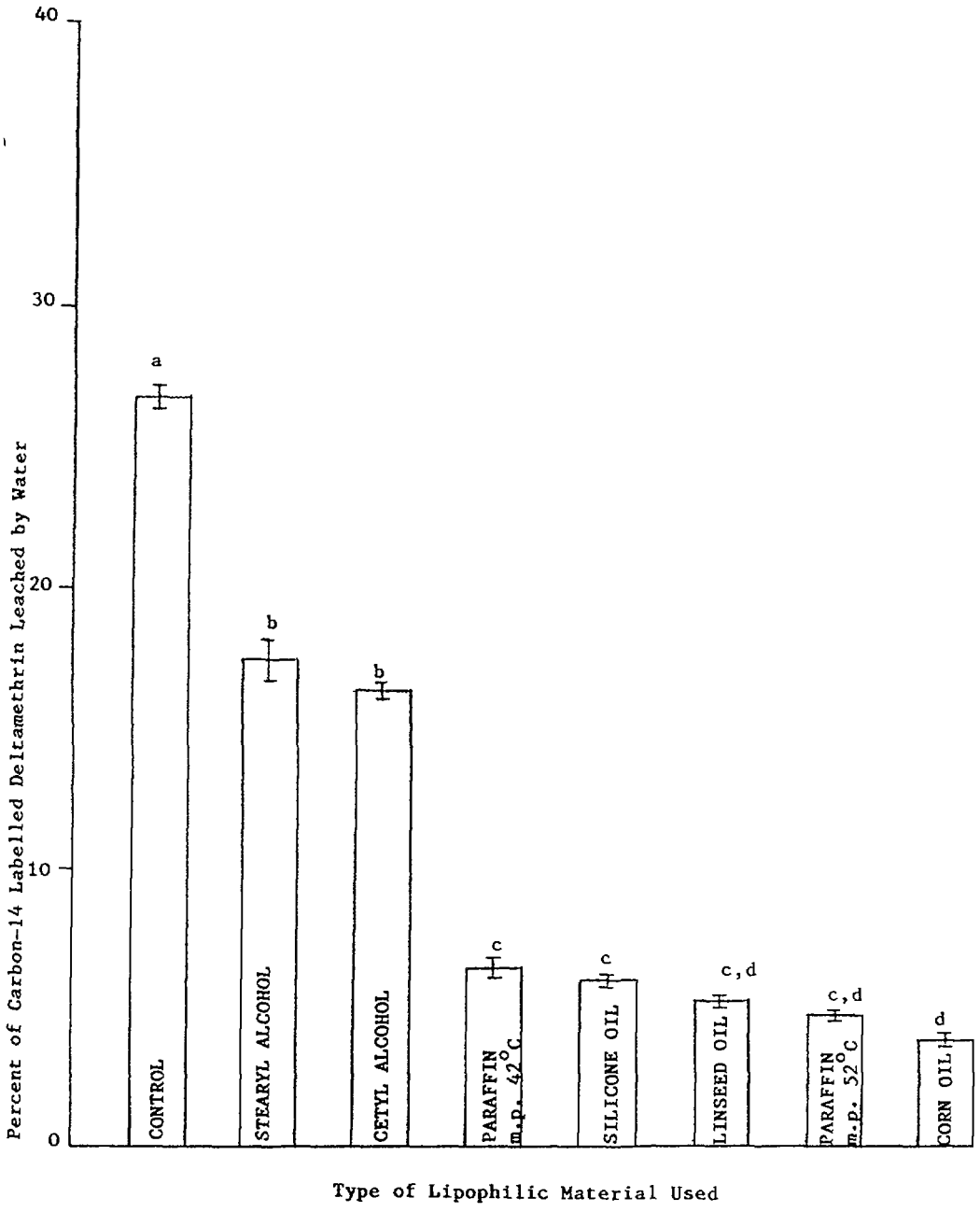


Fig. 2. The effect of different types of lipophilic materials on the leaching of deltamethrin by water from cotton fabric. Bars topped by the same letter are not significantly different (n=3, $P > 0.05$, Duncan's multiple range test).

Table 1. Percent of deltamethrin leached from cotton fabric in the presence of different type of lipophilic materials when subjected to successive washings with water.

Lipophilic material used	Percent of deltamethrin leached with successive washings				
	First Washing	Second Washing	Third Washing	Fourth Washing	Cumulative sum of four washings
Control	27.1 ± 1.2	4.1 ± 0.1	4.2 ± 0.1	2.3 ± 0.3	37.7 ± 1.7
Stearyl alcohol	17.8 ± 2.2.	4.6 ± 0.3	4.0 ± 0.2	2.8 ± 0.1	29.2 ± 2.8
Cetyl alcohol	16.7 ± 0.6	3.5 ± 0.4	4.4 ± 0.7	2.7 ± 0.7	27.3 ± 2.4
Paraffin (mp 42°C)	6.8 ± 0.8	2.2 ± 0.2	3.3 ± 0.2	1.9 ± 0.1	14.2 ± 1.3
Silicone oil	6.4 ± 0.6	2.4 ± 0.1	2.5 ± 0.4	2.3 ± 0.1	13.6 ± 1.1
Linseed oil	5.6 ± 0.6	1.9 ± 0.4	1.9 ± 0.2	1.9 ± 0.1	11.3 ± 1.3
Paraffin (mp 52°C)	5.1 ± 0.1	1.9 ± 0.1	3.0 ± 0.1	1.8 ± 0.4	11.8 ± 0.4
Corn oil	4.2 ± 0.4	2.0 ± 0.3	2.0 ± 0.3	1.7 ± 0.3	9.9 ± 1.3

The effect of fabric colour and/or UV absorber on the photodegradation of deltamethrin

Figure 3 shows that the colour of the fabric had a significant effect on the protection of deltamethrin from UV radiation. When the fabric was treated with deltamethrin alone (no UV absorber added) and irradiated under the UV lamp for 24 hours, very little (0.7%) of the applied amount of deltamethrin was recovered in the acetone extract from the white fabric. The extracts from the blue and black fabrics, on the other hand, contained 55.1% and 71.1%, respectively, of the applied amount of deltamethrin. This indicated that photodegradation of deltamethrin is greatly reduced on blue and black fabric.

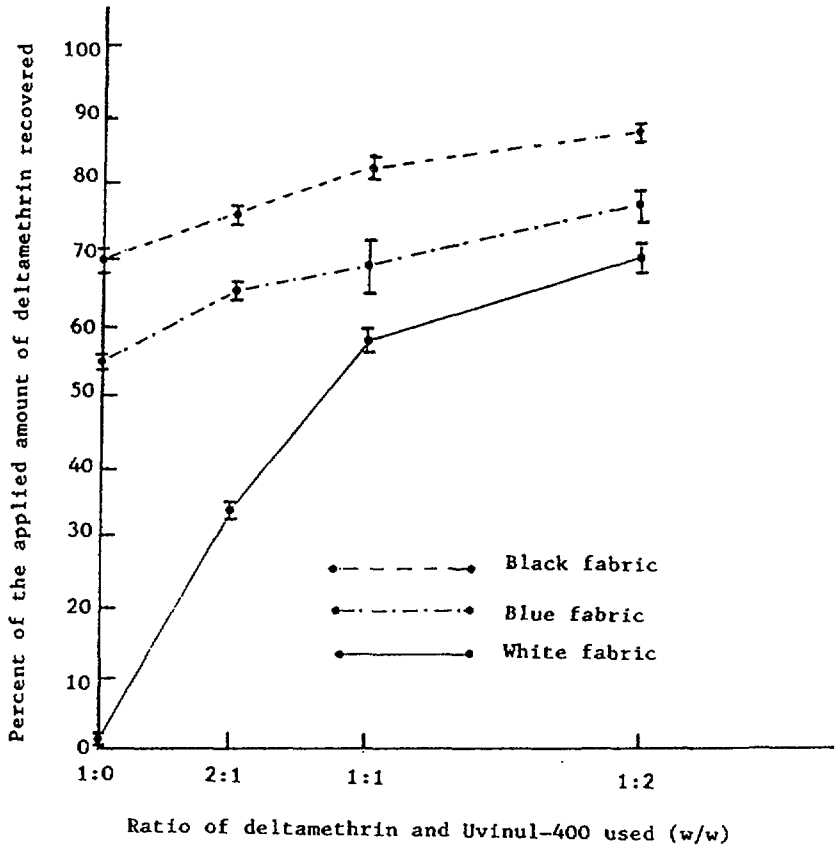


Fig. 3. Recovery of deltamethrin from cotton fabric of different colors after irradiation of the insecticide treated fabric under the U.V. lamp for 24 hours (n=3).

The addition of the UV absorber to deltamethrin on the fabric also resulted in protection of the insecticide from photolysis on white as well as blue or black fabrics. The degree of protection provided by the UV absorber was greater on white fabric than on the blue or black fabrics. Thus, when UV absorber was added to deltamethrin at 1:0.5 (deltamethrin: UV absorber, w/w) ratio, the extractable deltamethrin residues increased from 0.7 to 33.6% on white fabric, 55.1 to 65.2% on blue fabric and 71.1 to 76.1% on black fabric. Increase in the proportion of UV absorber compound resulted in an increase in the concentration of deltamethrin extracted from all three fabrics. However, this increase was greater for the white fabric than the blue fabric which was greater than that for the black fabric.

The effect of different periods of irradiation on the photodecomposition of deltamethrin applied with or without the UV absorber to the blue fabric is shown in Figure 4. Increasing the duration of exposure to the UV lamp resulted in a corresponding decrease in the amount of deltamethrin recovered. On the other hand, increasing the proportion of UV absorber to deltamethrin resulted in increasing recovery of deltamethrin from the fabric.

These data indicate that photodegradation of deltamethrin can be substantially reduced on cotton fabric of blue or black colour. The use of 2,4-dihydroxy-benzophenone as a UV absorber compound can further decrease the photodegradation of deltamethrin.

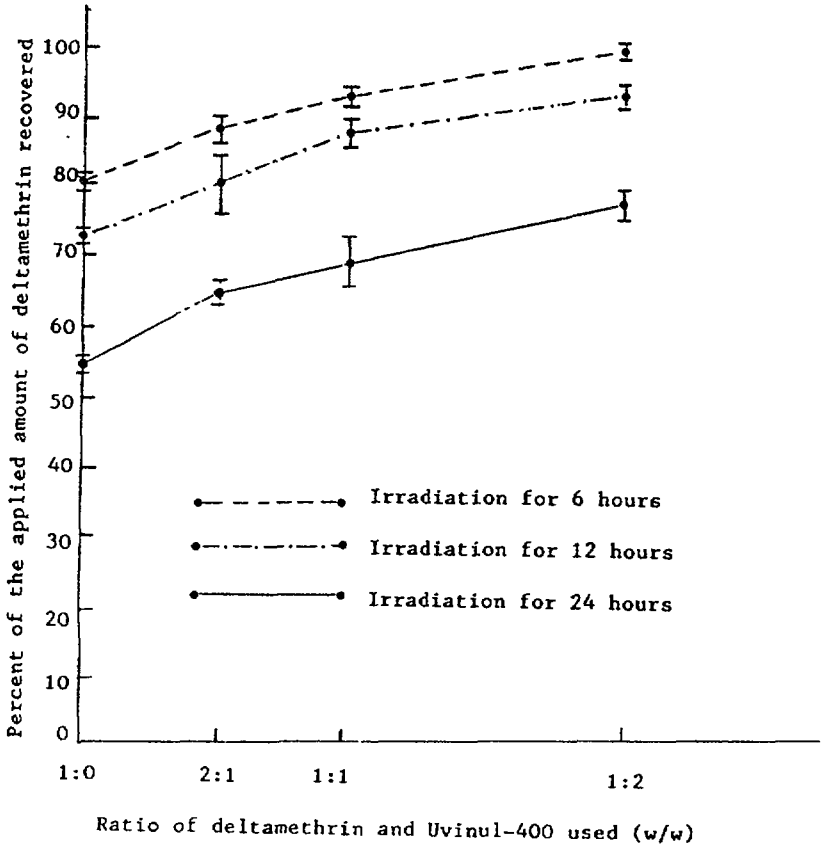


Fig. 4. Recovery of deltamethrin from blue cotton fabric after irradiation of the insecticide treated fabric under the U.V. lamp for different periods of time (n=3).

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EXPERIENCE WITH ODOUR BAITED INSECTICIDE IMPREGNATED TARGETS FOR CONTROL OF TSETSE FLIES IN KENYA

(Summary)

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Tsetse control in Kenya has hitherto been by use of insecticide sprayed from the ground supplemented by limited bush clearing in areas where human trypanosomiasis occurs. Animal trypanosomiasis is controlled by chemotherapy. Due to the occurrence of resistant trypanosome strains on Galana Ranch and in the coastal strip and the demonstration in Zimbabwe that odour-baited deltamethrin-impregnated targets were highly effective control devices against morsitans group tsetse flies. KETRI undertook to test the effectiveness of targets in the Kenya situation on the Galana Ranch. This was in an effort to find an additional trypanosomiasis control strategy. This trial began in January 1986 and lasted three years. The success of the trail resulted in a similar trial being undertaken in the Lambwe Valley. The Lambwe trial began in July 1988 and the objectives were (i) to evaluate the effectiveness and sustainability of deltamethrin impregnated odour baited targets and (ii) to involve the community in installation and maintenance of targets for their own protection.

On the Galana Ranch, four species of tsetse are found with Glossina pallidipes being the major vector. G. austeni, G. brevipalpis and G. longipennis are also present. The area in which the trial was conducted was a dense riverine bush bordered by bushed grassland spanning the Galana River. Targets baited with acetone and octenol were deployed at an average of 4 per km^2. Tsetse density was assessed using unbaited biconical traps for three weeks prior to installation, thereafter at three weekly intervals. Trypanosomiasis incidence was monitored in a herd of Boran cattle herded in the area with targets using the Berenil Index.

In the Lambwe Valley G. pallidipes is the only vector present. The area where the trial began is the Ruma National Park, an area of 120 km^2. Targets baited with acetone octenol and cow urine were installed after tsetse density monitoring using baited biconical traps. A herd of Zebu cattle grazing outside the National park is used for monitoring the incidence of trypanosomiasis in cattle. After the population of tsetse had been successfully controlled, barazas have been held with the local community to find out their attitude towards the current control taking place and their willingness to participate in the control.

On the Galana Ranch, a 99.95% reduction in trap catches was recorded in most areas with the central traps catching no flies. Glossina pallidipes was the first fly species to be affected followed by G. austeni. G. longipennis persisted. In the Lambwe Valley, the central area has remained free of flies two years from the time targets were installed. Traps sited in thickets near the border of the National park have continued to catch flies suggesting that there is reinvasion of the cleared areas by flies coming from the areas where targets have not been installed. These sites appear to follow the Olambwe river along which

very dense thickets are found. A reductin of 99.96% in the total trap catches has been recorded. There are plans underway to extend the trial to include the areas surrounding the National park. This will give a total area of 300 km^2.

On the Galana Ranch, trypanosomiasis in cattle remained very low and out of 180 animals there were 29 infections compared to 172 infections in 30 animals from an area where no control took place. The mean weekly PVC of the animals in both trials remained high during the control period except for periods when anaplasmosis infections were detected. In the Lambwe Valley, the Berenil Index dropped from 17 in September 1988 to 2 in 1989 and 0 by 1990.

It was observed that acetone evaporated much faster in the Galana situation. In the Lambwe Valley, the rate at which the clothes faded was faster for the clothes in the open as compared to those in the shade. While loss of target clothes in the Galana Ranch was a result of theft, bush fires was predominantly responsible for the loss in Lambwe Valley.

The local community in the Lambwe Valley appreciate the tsetse control being conducted in their area. The cattle keepers living adjacent to the game park reported improvement in the health of their animals and everyone recorded their willingness to participate in control using targets.

FIELD TESTING OF IMPROVED INSECTICIDE FORMULATION FOR CONTROL OF TSETSE USING BAITED TARGETS

(Summary)

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Field testing of insecticide impregnated blue strips for persistence began in Lambwe Valley in the middle of October 1990. Samples from the strips are cut every 20 days and are subjected to bioassays using teneral G. morsitans while samples are sent to Seibersdorf, Austria, for chemical analysis. The daily temperature and rainfall figures are kept for the period of the trial. To date, three samples, including the day 0 sample have been tested using teneral tsetse flies.

All tsetse flies that came into contact with the samples of treated strips cut on days 0,20 and 40 died, but after varying intervals. Observations are continuing.

DEVELOPMENT OF CONTROLLED RELEASE FORMULATIONS OF PESTICIDES USING NUCLEAR TECHNIQUES FOR THE CONTROL OF TSETSE FLIES

(Summary)

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Experiments were conducted on the use of controlled-release (CR) formulations of pesticides using nuclear techniques for the control of tsetse flies (Glossina spp.). Strips of cotton fabric screens impregnated with Carbon-14-labelled deltamethrin formulations, some of which contained oils, UV light absorber or both were tested. Then the strips were hung under tsetse habitats and tests were repeated at intervals of 2-3 weeks for about 6 months. Tests were of two types: Bioassay tests against tsetse flies to determine which formulations had the longest residual toxicity and radioanalytical test for determining radioactivity left on the strips and how it correlated with bioassay tests. Results show that total radioactivity and mortality decreased with time being rapid on unprotected strips or that containing oil. The strip containing UV light absorber or Glossinex had high losses of activity but continued to attain high mortalities up to day 175. Strip containing both oil and UV light absorber had the best performance. Until day 175 it recorded 85% mortality and radioactivity loss of 41% while the rest of the strips had lost more than 75%.

SIMPLE CREATION OF CONTROLLED RELEASE FORMULATIONS OF INSECTICIDES BY A POLYMERIC SPRAY TANK ADDITIVE

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Abstract

The seven main mechanisms whereby sprayed insecticides are rendered biologically inactive are identified. The beneficial diminution of these destructive effects by dissolution of the pesticide in a polymeric matrix is discussed. The problems of the controlled release delivery system so created and insecticide availability therefrom are analyzed in terms of the decreasing concentration of pesticide therein with the passage of time. The theory of a proposed method to circumvent the controlling laws of diffusion by dissolving the polymer in the insecticide, rather than vice versa, is explained and the idea of the addition of a bioinactive co-leaving component is introduced. The practicality of this new concept is demonstrated by the creation of a simple spray tank additive which forms a constant rate, polymeric, controlled release delivery system on the substrate sprayed. The functionality of the spray tank additive is exemplified by combination with three insecticides, methyl parathion, disulfoton and the toxin from Bacillus thuringiensis, and comparative biological testing.

Introduction

Insecticides begin to lose their effectiveness immediately after being sprayed into the environment due to volatilization, absorption by the substrate or plant sprayed, physical washing away by rain or dew, chemical degradation by combinations of oxygen, water, sunlight and/or micro-organisms. Soon the amount of insecticide remaining falls below the minimum level necessary to kill the insect pest and the end of the period of effectiveness of the insecticide has then been reached (1).

If the effects of these loss mechanisms are reduced, then the period of effectiveness of any given insecticide at a particular application level will be prolonged, and a more efficient use of the insecticide will thereby have been achieved.

One way of doing this is to dissolve the insecticide in a suitable polymer (2). Immediately, losses due to volatilization are reduced because the vapor pressure of the insecticide is lowered. Likewise, losses of the pesticide by absorption into the substrate or plant will be diminished because the partition coefficient between the solvent polymer and the underlying absorbent will favor retention of the insecticide in the polymer phase. Clearly, this retention will simultaneously diminish the opportunity for rain and dew to wash away insecticide since only material on the surface of the polymer will be susceptible to this physical removal. This will be a tiny

fraction of the total amount of bioactive chemical present within the polymer layer. Similarly, the polymer phase will tend to protect the dissolved insecticide from the degradative effects of oxygen, water, sunlight and micro-organisms by physically resisting contact with these entities. The minimized degradation caused by oxygen, sunlight and micro-organisms within the polymer phase can be further restricted by the inclusion of antioxidants, ultraviolet stabilizers and antimicrobial agents respectively.

Unfortunately, this approach has a very serious disadvantage stemming from the fact that the rate of escape of the insecticide from the protection of its polymeric prison is proportional to its concentration therein (3). This means that the amount of insecticide released to the surface, where it is available to kill the target insects, is inexorably diminished as time passes. The practical consequence is that the active level on the surface of the polymer phase soon falls below the minimum effective lethal concentration, even though the polymer phase still contains entombed a substantial fraction of the amount of insecticide initially present.

This shortcoming of the insecticide-polymer combinations can be rectified by the recognition that, as the proportion of insecticide to polymer increases, a point must be reached where the insecticide is no longer dissolved in the polymer, but rather the polymer can be instead considered to be dissolved in the insecticide. By definition, this would occur when the amount of insecticide exceeds the amount of the polymer phase (4).

Now, when the insecticide leaves, the polymer chains are too far apart to interact with one another to develop the interchain forces necessary to maintain the plastic structure. As a consequence, the polymer cracks, and flakes off, leaving the concentration of the remaining insecticide in the polymer unchanged. This constancy of concentration means that the driving force for insecticide release remains constant and a zero order rate of release is achieved.

Of course, under many situations, it is not desirable to have a formulation containing more than 50% insecticide because this can create problems in distributing or spraying the active pesticide over the desired area. For this reason, commercial insecticide formulations are usually put together so that the percentage of active ingredient is quite small, and is typically 10% or less. This difficulty can be resolved by including in the formulation a bioinactive leaving compound so that the sum of this and the insecticide is greater than 50%. When in use, both the insecticide and the leaving compound depart and the remaining polymer cracks and flakes off as before.

The leaving compound much be chosen with some care, and while many alternatives can be contemplated, the nontoxic and biodegradable polyethyleneoxyglycols offer many advantages. These materials are commercially available in a large range of molecular weights at a reasonable cost. This means that the rate of leaving can easy be slowed by selection of a higher molecular weight member of the group. Moreover, the polyethyleneoxyglycols are excellent solvents for most insecticides and polymers by virtue of the multiplicity of ether backbone linkages and their solvency in both water and organic solvents. Thus, compatible blends of polymer, insecticide and leaving compound can be easily prepared and the rate of insecticide release adjusted by either varying the amount or the molecular weight of the polyethyleneoxyglycol.

These blends can be conveniently prepared in the water in the applicator's spray tank by the simple addition of the insecticide, the leaving compound and the water-insoluble, rigid polymer phase in the form of a latex.

Both the insecticide and the leaving compound partition themselves between the water and the billions and billions of tiny polymer spheres with their enormous associated surface area.

When this mixture is sprayed onto a plant or substrate, the water evaporates and the insecticide, the leaving compound and the polymer sphere all merge together to form the controlled release blend containing more than 50% of the essential leaving entities, consisting, of course, of the insecticide and the polyethyleneoxyglycol.

A broad selection of bioactive chemicals has been successfully formulated in this system. Three examples will now be described to indicate the types of insecticides which can be handled, and which might be helpful for the control of the tsetse fly.

A controlled release formulation of the organophosphate, methyl parathion

Organophosphates constitute a large and powerful class of insecticides with the ability to control many pests economically. Decomposition of these chemicals often occurs rapidly into nontoxic residues that do not cause persistent contamination of the environment (5). Unfortunately, sometimes this decomposition is too swift to enable good control of the target insect to be achieved. For example, light sources of wavelengths 2537, 5889 and 6563 A at a temperature in excess of 37°C caused the breakdown of the insecticide, azinphosmethyl, under laboratory test conditions (6).

Through the courtesy of W.P. Scott, E.P. Lloyd and G.H. McKibben, Research Entomologists with the USDA, ARS, Boll Weevil Research Laboratory at Mississippi State University, a study of boll weevil control with controlled release formulations of this type was undertaken (7) for the suppression of the boll weevil, Authonomus grandis Boheman on cotton.

In this study, a standard methyl parathion spray formulation in the spray tank was admixed with a polyvinyl acetate-butyl acrylate copolymer latex (Ucar 368, 500g) and polyethyleneoxyglycol (Carbowax 4000, 110g) both donated by the Union Carbide Corp. and a ultraviolet light stabilizer (Tinuvin, 8g, Ciba-Geigy Corp.).

Four formulations were evaluated in which the ratio of polymers to active ingredient were stepwise increased. Formulation S was the standard methyl parathion water emulsion without the admixture of any controlled release polymers. Formulations A2:1, B4:3 and C1:1 contained one part of active ingredient in the methyl parathion water emulsion to 0.5 (2:1), 0.75 (4:3) and 1.0 (1:1) parts respectively of the Ucar 368-Carbowax 4000 mixture on a solids basis.

The test was conducted in a cotton field near Starkville, Mississippi, U.S.A., where randomly selected plots in the field were sprayed with each formulation at the rate of 0.5 lb of methyl parathion per acre. Immediately after the cotton plants had been sprayed, cages containing 10 laboratory-raised square-fed boll weevils were affixed to the stems of cotton plants in each of the treated plots. The cages were made from 0.5 pint ice cream cartons, the ends of which had been replaced with 16" mesh wire screens. The squares and leaves of the cotton plants were placed inside the cages so that the weevils could feed and would come into contact with the vegetation. This challenge was repeated 1, 3, 5, 7 and 9 days after the initial application of the insecticide formulations.

All spraying and subsequent caging of the weevils was completed within a period of two hours. The plots receiving the treatment with the standard formulation were the first to have the weevil cages attached and consequently the weevils in those plots were exposed to the insecticide slightly longer than the weevils exposed to the plants receiving treatments with the controlled release formulations.

The mortality of the weevils in all plots was measured 24 and 48 hours after each new exposure of weevils and the data collected were corrected by the use of Abbott's formula (8).

After the first exposure of the weevils to the treated cotton plants a small amount of rainfall (2.5 mm) occurred and immediately before the second exposure more rain fell (10 mm). During each of the next two days rain also fell, but the amount was too small to measure.

The brief duration of the period of effective toxicity of methyl parathion is evident in Table 1 from the mortality counts made 24 and 48 hours after attachment of the cages to the cotton plants. Acceptable weevil control was achieved for less than 1 day. In contrast, each of the controlled release formulations of methyl parathion gave excellent kills of the weevils for more than 7 days, a dramatic extension of the period of insecticidal effectiveness. However, formulations B4:3 and C1:1 were superior to formulation A2:1 and of this pair, the former might be said to have a slight performance edge over the latter. Accordingly, the recommendation for large scale field trials would be to add 3 parts of the polymer mix to the spray tank for every 4 parts of methyl parathion contained therein.

Table 1. Boll weevil mortality after prolonged exposure to controlled release methyl parathion formulations.

Days elapsed since application of formulation	Weevil exposure time, h	Boll weevil mortality on cotton after 24 and 48 h exposure to methyl parathion formulations*			
		S	A2:1	B4:3	C1:1
0	24	77%	100%	88%	95%
1	24	13	72	62	97
3	24	15	79	75	63
5	24	0	0	60	40
0	48	86	100	91	100
1	48	33	95	95	100
3	48	20	88	100	100
5	48	2	51	85	72
7	48	-	54	75	69
9	48	0	22	25	23

* S represents the standard methyl parathion formulation while A2:1, B4:3 and C1:1 are the controlled release formulations where the numbers 2:1, 4:3 and 1:1 indicate the ratio of methyl parathion to the polymer mix on a solid basis.

A controlled release formulation of the organophosphate, disulfoton

This controlled release technology is also applicable to situations where the insecticide is not sprayed onto the plant but is placed in the soil where it is absorbed by the roots and systemically translocated to kill insects feeding on the foliage (9). Usually, conventional formulations of insecticides do not provide a long enough period of effectiveness to control such pests and repeated treatments are mandatory.

An exemplar of this type of problem, which was brought to our attention by Professor H. E. Thompson of Kansas State University, is provided by pine tip moths (Lepidoptera; olethreutidae). These insects severely attack both ponderosa (Pinus ponderosa Laws) and Scotch (P. sylvestris L.) pines in Kansas (10). The control of such tip moths by systemic insecticides is not new and the effectiveness of many chemicals has been explored (11) for the suppression of the Nantucket pine tip moth in loblolly (P. taeda L.), slash (P. elliotii Engelm.), shortleaf (P. echinata Mill.), cluster (P. pinaster) and ponderosa pines. Trees protected from tip moth infestations exhibited significantly increased height growth relative to untreated controls but lost some of the increment when protection ceased and reinfestation occurred. Clearly, a prolongation of the period of effectiveness of the insecticide would be highly desirable.

One of the insecticides selected for study was the organophosphate, disulfoton, supplied by the Chemagro Corp. (Kansas City, Missouri 64120) as a 23% (w/w) solution of technical material. This was admixed with the polymer mixture described above so that the ratio of pesticide to polymer was 1:2. The blend was diluted with an equal volume of water and dispensed into the planting medium around the roots of the planted pines by the use of an automatic pipette.

The plants used in the test were two-year-old ponderosa (90 trees) and Scotch (90 trees) pines, individually growing in rectangular isolation tarpaper tubes (5 x 5 x 20 cm) containing a mixture of soil and peat. These were transplanted 30 cm apart in 5 replicated separate blocks of 3 rows of 3 seedlings into a sandy loam field near Junction City, Kansas. Each block of 9 trees received either 0, 0.5, 1.0 or 2.0 g of active ingredient/tree so that a total of 45 trees comprised each treatment level.

All seedlings were treated with the controlled release insecticide formulations in the Spring at the end of May and the number of infested terminals and lateral shoots in each replicate was recorded in mid-September of the same year and at the end of May of the following year, which was one year after the initial treatment. Similar data was gathered at the beginning of July of the second year and at the end of May of the following year, when the experiment had been underway for two years. To stabilise the variance, the data gathered were first transformed by the use of the relationship, $Y = \sqrt{(count + 1)}$, and thereafter statistically treated by using a combination of a SAS analysis of variance and Fisher's t-test to determine differences at the 95% confidence level.

The trees used in this research were transplanted just before the usual time for apical shoot elongation. However, elongation was delayed, presumably by the trauma of transplantation, and since pine tip moths only attack new growth, infestation of the seedlings did not begin until the genesis of the second generation in July. Thereafter, as the results collected in Table 2 show, at the 95% confidence level, that untreated seedlings of both species were atacked by the three insect generations which appeared during the second year. Moreover, at the 95% confidence level, the data in Table 1 indicates

Table 2. The extent of pine tip moth infestation of Scotch and ponderosa pines at various elapsed times after a single treatment with a controlled release formulation (CRF) of the insecticide, disulfoton.

Pine species	CRF-Dosage* (g a.i./tree)	Number of pine tips infested after 4 months	1 year	14 months	2 years
Scotch	zero	7	17	7	18
	0.5	2	5	6	19
	1.0	0	0	0	5
	2.0	0	0	0	5
Ponderosa	zero	16	9	5	8
	0.5	0	2	0	3
	1.0	0	0	0	3
	2.0	0	0	0	2

* The effectiveness of the 1.0 and 2.0 g treatments are about equal, but both are more effective than the 0.5 g treatments at a confidence level of >95%.

that P. sylvestris is somewhat more susceptible to attack than P. ponderosa throughout the entire period of the experiment. However, this susceptibility can be remedied by the controlled release combinations.

At the highest treatment level, the formulation remained bioactive for at least 14 months from the date of initial application. It should be noted that the next to highest level of insecticide can be statistically regarded as equally effective at the 95% confidence level. Sometime later, but before two years elapsed, the protection provided by both the 1 and 2 gram levels began to fade.

It is to be emphasized that Barras, Clower and Merrifield (12) were able to secure protection from tip moth attack during the last three generations in 1960 and the first generation in 1961, by the application of conventional commercial granular formulations of phorate. Although granular materials often exhibit, to a degree, some controlled release characteristics by virtue of their geometry the quantities of active insecticide necessary had to be augmented to 8.4g active ingredient per tree to achieve the extended period of protection. This amount of pesticide is many times greater than the quantities (1 or 2g) found to be effective in this study. Thus, once again, the intrinsic economic and environmental advantages of this polymeric spray tank additive system are demonstrated.

Of course, the polymeric spray tank additive system can be applied to insecticides other than organophosphates and good results have been obtained with other types of pesticides, including the bacterial material from Bacillus thuringiensis.

A controlled release formulation of the toxin from Bacillus thuringiensis

Through the courtesy of W. McLane of the U.S.D.A. a number of laboratory tests were conducted with a commercial form of the toxin of Bacillus thuringiensis (Dipel 8L, Abbott Laboratories, N. Chicago, Illinois) using newly moulted second instar gypsy moth larvae as the target test insects. The gypsy moth is an important forest pest in the U.S.A. In this set of experiments the spray additive system was a commercial version currently offered under the name HOLDUP (Greenshield of Washington, P.O. Box 82622, Kenmore, WA 98028).

Tender red oak (Quercus rubra L.) seedlings were sprayed in a laboratory chamber with Dipel 8L alone and admixed with various amounts of HOLDUP. After the treated foliage had dried for 2 hours, twenty 2nd. instar laboratory reared gypsy moth larvae were then allowed to feed on each seedling. Each test was replicated 5 times while the test seedlings were held at 80 F and 60% relative humidity. A treatment standard and an untreated check were part of each individual test.

The results obtained and collected in Table 3. show that the bacterial insecticide in combination with HOLDUP consistently provided a greater degree of control than the insecticide alone. HOLDUP itself can be seen to have no insecticidal properties. The 1:1 ratio of Dipel 8L to HOLDUP appeared to be the most effective formulation at a dosage of 12 BIU/acre. The addition of HOLDUP to the Dipel 8L insecticide also had a beneficial effect in reducing the amount of defoliation due to insect attack.

Table 3. Oak seedling defoliation and 2nd instar gypsy moth larvae mortality after prolonged exposure to controlled release Dipel 8L formulations.

HOLDUP addition*	Dosage BIU/acre	Percent defoliation days from exposure 2	3	4	Percent mortality days from exposure 2	3	4
0	6	32%	50%	60%	0%	1%	25%
3.5	6	13	17	24	24	41	66
7	6	5	5	5	54	77	91
0	8	8		33	17		50
1	8	8		38	28		64
0	12	1		20	35		86
1	12	1		3	73		97
2	12	2		8	52		89
0	12			33			78
0.1	12			38			68
0.25	12			31			79
0.5	12			17			93
1.5	12			7			96
0	12	8	20	38	13	36	65
1.5	12	4	6	8	46	71	88
3	12	9	12	15	33	57	78
0	12		25	40		41	59
1.5	12		3	3		83	95
3	12		6	6		81	94
HOLDUP alone				100			0

* The HOLDUP addition numbers refer to the amount of HOLDUP added to one part of Dipel 8L. For example, HOLDUP addition number 1.5 means that 1.5 parts of HOLDUP was mixed with one part of Dipel 8L and then sprayed on the oak seedlings.

Note: Untreated controls were used with every test in order to have a background check. In all cases defoliation was nearly 100% and mortality was near 0%.

For this case, it is highly unlikely that the active principle from Bacillus thuringiensis is a solvent for the controlled release polymer. More probably, the insecticide is simply encased within the mixture of the water-insoluble and water-soluble polymers and becomes sequentially accessible to the larvae as the latter erodes and exposes the toxic material.

Conclusion

A new and practical method of extending the effective life of a variety of pesticides by the simple addition of a water-based erodible polymer mixture to the spray tank has been discovered and biologically tested. The polymer mixture protects the pesticide from the deteriorating forces of nature. The system is advantageous environmentally because it enables readily biodegradable entities to be used in place of the more persistent chemicals which are often presently employed. It should be applicable to the improved chemical control of the tsetse fly and may permit the application of insecticides currently not being considered because of their fugitive characteristics or cost.

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TSETSE AND TRYPANOSOMIASIS CONTROL IN ZIMBABWE

(Summary)

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Trypanosomiasis, a disease transmitted by tsetse flies, is a major constraint on Agricultural Production in Tropical Africa.

In Zimbabwe where agriculture, and particularly stock raising, is of major economic and social significance, the control of the tsetse fly leading to its eradication is a task of national importance.

Zimbabwe has achieved significant progress in tsetse and trypanosomiasis control with a total of 48,000 km^2 being cleared of tsetse since 1980. The cleared areas have been consolidated by planned human settlement whose agricultural production has increased following introduction of cattle which provide much needed traction power.

Tsetse flies are now confined to an area of 25,000 km^2 along the Zambezi Valley.

Several strategies are being used to control tsetse flies with the latest technique being the use of odour-baited insecticide-impregnated targets. When used at a density of 4/km^2, targets have been found to be extremely effective against the species of tsetse flies found in Zimbabwe.

The work undertaken in Zimbabwe as well as prospects for the future within the EEC-funded Regional Tsetse and Trypanosomiasis Control Programme will be discussed.

INTEGRATED TSETSE MANAGEMENT IN SOUTHWESTERN ETHIOPIA

(Summary)

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In recent years the food crisis has been a burning issue on the African continent. Many factors have been associated with low levels of food production including drought, harsh and unproductive environments, disease and mismanagement. In Ethiopia, about 85% of an estimated population of 45 million are employed in the rural agricultural sector. The birth rate is estimated at 2.9% per annum. This increase in human population has resulted in intensive land use pressue and the adoption of exploitative farming pressure with consequent land use degradation. Most of the ecologically fragile areas are to be found in the highlands and contain 92% of the human and 78% of the livestock populations of the country. The most obvious option for future agricultural development are the lowland areas of western and southern parts of Ethiopia. These areas are infested by tsetse and the associated disease trypanosomiasis. According to the report (MOA, 1990), the utilization of tsetse infested areas with high potential for agricultural production has been a major priority of the government of Ethiopia. This has resulted in the development of state farms, first introduced in 1974, and agricultural settlement schemes, especially after the 1984/85 famine in the western part of the country. In order to try and overcome the constraints caused by the presence of tsetse-transmitted trypanosomiasis, farms and settlement schemes initially relied heavily on mechanized cultivation. Serious problems with servicing of machinery and associated problems in getting the necessary spare parts has meant that the experience with mechanization has been far from satisfactory. As a result, the intention is to shift the emphasis to more traditional systems of agricultural productin relying on animal drought power. This can only be achieved, however, if the problem of tsetse-transmitted trypanosomiasis can be alleviated.

In view of this, a collaborative research programme has been conducted in order to develop a practical tsetse management model which combines cost effective tsetse control method, strategic use of drugs and environmentally sound land use planning. In this connection, special efforts have been made to validate and modify the already known tsetse control techniques and some of the results are indicated below. The NGU trap baited with acetone and cow urine showed significant increases (4.8-fold for male flies and 7.5-fold for female flies) over the unbaited bioconical trap. The biconical traps baited with cow urine, hippo dung and Octenol yielded highter catches (1.3 to 2.6-fold increase for male flies, 1.4 to 4.6-fold for females) than unbaited bioconical traps. Cow urine, however, was the only one of these attractants that showed a significant increase in most instances. Biconical traps baited with pig urine and acetone showed a significant 2.6-fold increase over unbaited biconical traps.

A new trap design (Tetra-trap) is tested against biconical and NGU traps. Cow urine, acetone and a 4:8:1 mixture of octenol, methyl phenol and propyl phenol obtained from the Tropical Diseases Research Institute was tested using the new trap design.

The index of increase of the tetra trap baited with pnehols over the unbaited biconical is about 8, over the baited biconical trap is about 4 and over the baited NGU trap is about 2. Comparison of acetone, cow uring and acetone, octenol and acetone and an unbaited control showed that while the acetone alone did not increase the catch significantly, both the cow urine and acetone and the octenol and acetone increased the catch by 2.5 to 3 times.

INTERIM STRATEGIES TO CONTROL ANIMAL TRYPANOSOMIASIS IN TWO SELECTED VILLAGES ALONG THE WHITE VOLTA RIVER IN THE ONCHOCERCIASIS FREE ZONE OF NORTHERN GHANA

(Summary)

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The onchocerciasis eradication campaign in the west African subregion was a remarkable success which made formally deserted lands now suitable for settlement. Recent tsetse and trypanosomiasis surveys along the White Volta river indicate that livestock development would virtually be impossible in these areas without some form of intervention to contain animal trypanosomiasis. Apparent fly densities are in the order of 5-10 flies/trap/day (biconical traps) with fly infection rate being in the order of 1-8.0%. Trypanosomiasis is particularly prevalent in small ruminants (12-30%) with several reported cases of abortion. In view of the sparse human population in some of these areas, large scale control programmes would not be advisable unless there is a clear land-use plan. In the interim, however, efforts could be made to control the disease at the village level.

This paper outlines some village-based strategies that could be adopted by settlers in these areas.

TRYPANOSOMIASIS CONTROL AND ERADICATION OF *Glossina austeni* FROM ZANZIBAR USING AN INTEGRATED APPROACH

(Summary)

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Eradication of G. austeni for the removal of the trypanosomiasis constraint from the island of Unguja, Zanzibar, appears feasible by using an integrated approach.

The use of animals as living baits, treated with a 1% deltamethrin pour-on oil formulation, combined with drug treatment of animals found infected with trypanosomes was successful in grazing areas with a sufficient number of cattle.

In areas where other tsetse hosts are present or where cattle are absent (like Jozani and Muyuni forests), stationary targets impregnated with Alfacypermethrin 10% E.C. were found effective in reducing fly populations. Such prior suppression is suitable before the release of sterile males.

In order to enhance the future use of the SIT where cattle treatment or stationary targets alone may not result in tsetse eradication, pre-release studies on transportation and deployment of sterile flies produced at Tanga and released in a related block of the dense forest have commenced recently, as well as observations on the G. austeni populations structure.

Results of work on the bait technology and investigative work using sterile flies are described in the papers of D. Höreth-Böntgen and M. Vreysen et al.

CONTROL OF *Glossina austeni* AND CATTLE TRYPANOSOMIASIS IN UNJUGA ISLAND BY DELTAMETHRIN POUR-ON APPLICATION TO LIVESTOCK AND WITH STATIONARY TARGETS IN CATTLE-FREE ZONES

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Abstract

Tsetse control with odour-baited artificial targets has been widely used in recent years in countries like Zimbabwe, Zambia, Ethiopia, Ivory Coast and Burkina Faso among others. More recently, this method was expanded by the use of the host animal itself, as a "living target". The development of highly effective synthetic pyrethroids in long-lasting formulations, and particularly the low mammalian toxicity of these compounds, made such an approach possible.

The first trials have been carried out in Zimbabwe, Zambia, Zanzibar and recently at Mkwaja Ranch in Tanzania. Results achieved with FAO assistance in a pilot-trial in the Mangapwani area on Zanzibar have been converted into a large-scale operation under the UNDP/FAO supported project URT/86/022, aiming at complete removal of G. austeni from the island of Unjuga.

The present paper outlines the results achieved so far with the use of sequential, topical application of deltamethrin on cattle, goats and donkeys to eradicate G. austeni. An alternative approach for tsetse control in areas where cattle are virtually absent is also described.

Introduction

Research on the behaviour and ecology of various tsetse species has led to the development of odour-baited, stationary targets, as an environmentally safe control method. Most work has been carried out in Zimbabwe (Vale, 1985; Vale et al., 1988).

The development of synthetic pyrethroid insecticides and their successful use for tsetse control, first during spraying operations (Spielberger et al., 1979) and later applied to artificial targets (Laveissière et al., 1981a, b and Küpper, 1985), has finally led to the treatment of domestic animals, thus taking profit of their natural attractivity to tsetse flies.

Treatment of cattle with a deltamethrin-based acaricide for controlling tsetse was first done in Zimbabwe. In Zambia deltamethrin was nearly simultaneously used in splunge dips. In both circumstances, a tremendous decline of the apparent fly population and a marked reduction in trypanosomiasis transmission was observed (Hursey et al., 1987; Wilson, 1987 and Chizyuka et al., 1986). Work in Zimbabwe and Zambia was followed by a pilot trial with deltamethrin as a pour-on formulation under the FAO project TCP/URT/6758 in Mangapwani area on Zanzibar (Schönefeld, 1988) and by a trial with deltamethrin in a dipping-formulation at Mkwaja Ranch in Tanzania (Fox et al., 1990).

The results of Schönefeld's pilot-trial formed the basis for a large-scale operation in the framework of the UNDP/FAO Animal Disease Control Project URT/86/022, implemented in co-operation with the Livestock Development Department in Zanzibar. Achievements obtained so far are highlighted in this paper.

Materials and Methods

Since the successful completion of the Mangapwani pilot trial in May 1988, a well-known, heavily tsetse-infested area of roughly 33 km^2, 4 control blocks joining the pilot trial area to the east and south have been established. So far, they cover roughly 600 km^2: Block A (76 km^2); Block B (95 km^2); Block C (230 km^2) and Block D (195 km^2).

Cattle, goats and donkeys in each block are regularly treated externally with deltamethrin-acaricide as a pour-on formulation (SPOTON, Coopers, Zimbabwe). The insecticide is applied with a specially designed, calibrated applicator at a dose-rate of 10 ml/100 kg bodyweight. Calves below the age of 6 months are exempted from it, to allow for the build-up of a natural immunity against prevailing tick species. A campaign includes 7 cycles: the first 5 cycles are spaced at a fortnight's interval; the two last cycles are given at 3 weeks interval. In overlapping areas, a "barrier-treatment" is continued for some more cycles to prevent re-invasion until the next block is under treatment.

Prior to the establishment of a new block, pre-treatment surveys for tsetse flies were carried out with "sticky 3-dimensional-targets". Success monitoring is carried out continuously in the same way. Cattle found positive with T. congolense, T. vivax or a combination of both, are curatively treated with diminizene aceturate (Berenil^R - Hoechst, FRG), at a dose rate of 5.25 mg/kg liveweight.

In cattle-free areas, tests have been initiated out to find an effective alternative to living targets, on the basis of stationary targets. This method is presently tested on a larger scale in the Jozani forest. Cloth screens of roughly 1 m^2 of either blue or white colour are suspended close to the ground and impregnated with alphacypermethrin at 0.05% working-solution, prepared from an emulsifiable concentrate of 10% a.i. Fendona (Shell, UK).

Results

Compared with Mangapwani, Blocks A, B and C had only a low density of tsetse flies, due to different environmental conditions. Mangapwani is a tree-cropping area (clove and coconut) with dense understored thicket-vegetation. Here, the untreated G. austeni population did find suitable habitat conditions, with easy access to free-grazing cattle. In the blocks to the south, the situation is completely different. There is either intensive agriculture with cassava and rice plantations or coral-rag areas, where tsetse flies were confined to isolated thicket fringes and with cattle kept at safe distance from the cultivated areas. Therefore, fly/domestic host contact was less intimate. For this reason, the standard treatment period was extended with two more cycles.

Fly Density

The presence and relative abundance of G. austeni was assessed making use of sticky panels. Pre-control monitoring in the various blocks resulted in the following catches: for Mangapwani, 127 flies at 26 sites, for Block A, 8

flies at 4 sites, for Block B, 3 flies at 3 sites and for Block C, 6 flies at 3 sites. The situation in Block D, with the Jozani forest in its centre as the main focus, is different. Outside the forest reserve, 83 flies were caught at 10 sites, whereas 31 panels positioned in the southern half of the real forest yielded 2013 flies during the period 6 October 1990 to 2 February 1991.

Trypanosomiasis Incidence

Pre-control trypanosomiasis surveys gave the following picture for the animals examined:

In Mangapwani: 217 positives or 46% out of 472 cattle bled, with 119 cases or 55% of T. congolense, 80 cases (37%) of T. vivax and 18 cases (8%) of mixed infections.

In Block A: 112 positives or 18% out of 627 cattle bled; 32 (29%) T. congolense, 62 (55%) T. vivax and 18 (16%) mixed infections.

In Block B: 66 positives or 14% out of 475 cattle bled; 41 (62%) T. congolense, 16 (24%) T. vivax and 9 (14%) mixed infections.

In Block C: 79 positives or 11% out of 713 cattle bled; 26 (33%) T. congolense, 39 (49%) T. vivax and 14 (18%) mixed infections.

In Block D: 121 positives or 28% out of 429 cattle bled: 65 (54%) T. congolense, 40 (33%) T. vivax and 16 (13%) mixed infections.

In these blocks, the average incidence is not reflecting the reality, as trypanosomiasis is generally high in sectors close to locations where flies have been caught and is diminishing away from those places. The highest incidence in Block A was found in Ghana (37%) and in Bumbwisudi (35%) in the immediate vicinity of tsetse catching sites. In Block B, Kiboje with 51% had a much higher incidence than all other sectors. In Block C, infection rates in animals grazing close to the 3 catching sites ranged from 18% to 21%. In Block D, cattle at the periphery of the Jozani forest showed infection rates from 34% to 56%, with far fewer cases away from it.

Cattle Distribution in the Blocks

In the Mangapwani pilot trial area 695 cattle, 200 goats and 20 donkeys have received regular deltamethrin treatment. Related to the size of the area (33 km^2), this represented 28 "living targets" per km^2.

This coverage continuously decreased for the other blocks. In Block A (76 km^2), 1076 cattle, 148 goats and 15 donkeys on average have been treated, resulting in a "living target" density of 16; in Block B (95 km^2), with 1389 cattle, 248 goats and 35 donkeys treated, the density was 18; in Block C, the largest block so far (230 km^2) with 2128 cattle, 262 goats and 1 donkey treated, a target density of 10 per km^2 was reached. Finally, in Block D (195 km^2), with 1200 cattle, 450 goats and 1 donkey treated, a density of 9 animals per km^2 was reached. This is just about double the coverage used in savannah conditions with stationary targets at optimum lay-out.

Control Achieved

In Mangapwani, where all cattle were given a blanket treatment of Berenil, no positive cases were detected after completion of the pilot trial.

Fly monitoring also did not result in any catch at previously good catching sites.

In the other blocks, no blanket treatment was given due to the much higher number of cattle and the low trypanosomiasis incidence. This would have been a waste of a very expensive drug. Therefore, positive animals are still found on occasion of trypanosomiasis surveys carried out in the previous blocks. However, case history always indicated that the cattle were either bled for the first time or that the last bleeding was done when treatments in the blocks were still underway. This leads us to believe that in these areas, G. austeni has been successfully controlled, as no flies were caught at reference and occasional sites after the completion of the treatment cycles.

Only in Block D the situation was different. Here, positive cattle were detected continuously. The first breakthrough was just before the 12th treatment cycle, when bleeding of cattle in the whole area revealed only 1 positive animal which was found infected before, and 6 other positives among animals bled for the first time.

The reason for this low level persistence of infections is the presence of flies in the nearby Jozani forest. The fly population here is not affected by the deltamethrin treatments of cattle, as the forest is mostly impenetrable for domestic animals. Moreover, the forest is full of natural host animals (bushpigs) which enable G. austeni to feed and sustain its lifecycle. To overcome this situation, a trial was started at the beginning of October 1990 with stationary, artificial targets made of cloth-screens and impregnated with cypermethrin.

In a first phase, 12 transects of 800m were cut into the forest reserve in west/east direction. Transects are 100 meters apart and cover an area of approximately 1 km^2. In each transect, 3 cloth-screens impregnated with 0.05% alpha-cypermethrin were placed at 200m interval in an interlocking pattern of white or blue colour, together with a set of "3-dimensional sticky targets". As shown in Table 1, the catches declined markedly within 3 weeks.

Table 1 Comparison of G. austeni catches (average number of flies per target per day) for targets placed close to the forest edge and those placed inside the forest (Phase 1)

Date	**6 Oct**	**8 Oct**	**11 Oct**	**12 Oct**	**13 Oct**	**15 Oct**	**17 Oct**	**20 Oct**	**22 Oct**
Forest edge	0.00	5.17	1.76	2.00	4.00	1.61	1.39	0.56	0.78
Inside forest	1.25	3.00	2.25	16.30	7.00	3.43	2.36	2.29	1.07
Date	**27 Oct**	**29 Oct**	**31 Oct**						
Forest edge	0.69	0.89	0.36						
Inside forest	0.80	0.79	1.42						

In a second phase, the area has been extended by the introduction of 3 more transects in the north and in the south. The 2 northern transects, stretching 2.5 and 1.5 km into the forest, contain 25 screens (100m apart) and 7 sticky targets spaced by 200m and act as a barrier against flies in the untreated northern half of the forest. Again, in the beginning, high catches were recorded in the new transects followed by a marked decline after 3 weeks (Table 2). The 18 transects presently contain 82 cloth-screens and 25 "sticky targets". In the central part, catches remained low, although the set-up is open to re-invasion from the untreated east. The first phase started at the beginning of October 1990 and was followed by phase 2 one month later. Up till now (February 1991), the cloth-screens have been resprayed 3 times, to compensate for the losses of insecticide due to rainfall in previous months. The monitoring "sticky targets" have been replaced twice, resulting everytime in a slight increase in fly-catches over a week's period.

Table 2. Comparison of G. austeni catches average number of flies per target per day) for targets placed close to the forest edge and those placed inside the forest (Phase 2)

Date	**3 Nov**	**6 Nov**	**10 Nov**	**15 Nov**	**17 Nov**	**21 Nov**	**26 Nov**	**29 Nov**	**1 Dec**
Forest edge	2.64	1.09	1.67	0.32	0.71	0.08	0.17	0.03	0.00
Inside forest	4.81	2.51	2.63	0.74	1.00	0.57	0.37	0.33	0.34
Date	**5 Dec**	**8 Dec**	**13 Dec**	**15 Dec**	**17 Dec**	**24 Dec**	**27 Dec**	**29 Dec**	**1 Jan**
Forest edge	1.13	0.56	0.43	0.13	0.13	0.05	0.22	0.19	0.50
Inside forest	0.10	0.12	0.11	0.03	0.10	0.08	0.04	0.00	0.47
Date	**5 Jan**	**10 Jan**	**15 Jan**	**21 Jan**	**23 Jan**	**25 Jan**	**29 Jan**	**4 Feb**	
Forest edge	0.33	0.13	0.11	0.09	0.00	2.72	1.72	0.19	
Inside forest	0.43	0.23	0.20	0.10	0.07	1.31	1.88	0.57	

In a third phase, all transsects will be extended through the entire forest and the number of cloth-screens will be accordingly increased.

Discussion

The results achieved so far under TCP/URT/6758 and URT/86/022 are in line with tsetse-control operations under similar conditions in other countries like Zimbabwe in the Rifa triangle (Vale et al., 1988) and in the Didessa valley in Ethiopia (Slingenbergh, 1990), where odour-baited artificial targets were achieving 99% reduction in apparent fly density.

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LABORATORY AND FIELD OBSERVATIONS IN RELATION TO THE RELEASE OF STERILE *Glossina austeni* ON UNJUGA (ZANZIBAR) ISLAND

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Abstract

The radiation treatments given to male Glossina austeni before their deployment in the field had no detectable effect on viability, insemination potential or competitiveness. Excellent results were obtained for both treated and untreated control males at cage densities of up to 800 flies.

A total of 22,563 male G. austeni were transported from TTRI, Tanga, to the Jozani forest, Unjuga island, under two different release schedules. On average, 91.6% of the males transported were actually released. Direct transport in the morning (average transport time was 2.5 ± 0.5 hours) proved to be superior (release rate of 94.7%) compared to a schedule in which flies were transported from Tanga to Zanzibar in the evening with the release being done the next morning (release rate of 85.8%).

Pre-release studies in the northern Jozani forest, demonstrated the efficiency of the sticky panel as a monitoring device for the collection of baseline data on apparent densities and age structure of the G. austeni population.

Sterile female G. austeni were also released in the middle of the experimental block to test their usefulness as tracer insects. Treated females recovered 5 or more days post-release, were all found to be inseminated by native males.

Introduction

Tsetse-transmitted trypanosomiasis is one of the major factors limiting livestock production in the coastal regions of mainland Tanzania and on Unjuga island of Zanzibar. In close cooperation with the national authorities, the IAEA has initiated a technical assistance project with the objective of contributing to the control or eradication of tsetse flies. Priority has been given to the situation on Zanzibar, where Glossina austeni is the only tsetse species present. For this reason, the sterile insect technique has been introduced as part of an integrated management plan in which the FAO plays a key role.

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The Tsetse and Trypanosomiasis Research Institute (TTRI) at Tanga, is mass-rearing G. austeni by means of the artificial membrane feeding system for the production and sterilization of male flies. The Tsetse Control Unit of the Department of Livestock Development on Zanzibar, in collaboration with FAO field staff, has already collected valuable ecological and entomological data related to tsetse population suppression by means of stationary and living targets, as well as data on trypanosome infection rates in domestic animals.

In preparation of the sterile male release programme, studies were conducted first at the Tanga laboratory and subsequently in a selected area of Zanzibar island, to assess the effect of fly handling (i.e. radiation treatment, packaging of treated flies at various cage densities and their transport from the rearing facility to the natural habitat) on important biological parameters. Additional information was collected on fly abundance and population structure in a defined sector of the Jozani forest. Before starting experimental releases of sterile males, a small field experiment was conducted with radiation-treated female G. austeni to evaluate their potential use as sentinel or indicator insects for future monitoring purpose. Results of these experiments are reported hereinafter.

1. Laboratory Studies

1.1 Materials and Methods

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

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

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

1.2 Results

(i) The average lifespan and trends in longevity for control males, for colour-marked males, for 120 Gy-treated males and for marked and radiation-treated males is given in Table 1. Mortality, due to the various treatments, was within acceptable limits. All types of matings resulted in normal insemination and normal fecundity except for females (i.e. no pupa production) mated to the radiation-sterilized males.

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

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

Treatment	Average lifespan (Mean ± SD.) (days)	Days following emergence to reach indicated % mortality		
		50	90	100
Control	76.7 ± 30.0	91	98	99
Marking	66.8 ± 36.4	89	97	99
120 Gy Ir.	52.2 ± 20.2	54	71	96
Mark./120 Gy	58.4 ± 24.2	60	90	98

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

No. males in release cage	Treatment	Transport	% Survival on indicated days following emergence			
			10	20	30	40
300	Control	none	96.6	93.3	81.3	68.7
300	Marking/120 Gy	none	93.4	86.2	60.6	65.8
500	Control	none	96.0	92.6	86.4	78.3
500	Marking/120 Gy	none	89.6	85.7	80.6	66.2
500	Control	3 hours	98.1	91.5	85.3	71.7
500	Marking/120 Gy*	3 hours	93.0	88.7	83.2	70.2
500	Marking/120 Gy**	3 hours	97.4	93.6	78.7	59.6
800	Control	none	97.0	94.4	90.0	86.7
800	Marking/120 Gy	none	90.0	88.1	77.4	66.3

* Marking and irradiation treatment on the same day.
** Marking and irradiation treatment on 2 consecutive days.

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

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

1. 2 x 30 males in container with 20 virgin females being added.

Table 4.: Competitiveness of marked/irradiated G.austeni males kept at a density of 500 / release cage and transported for 3 hours. 1/

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

1. 2 x 30 males in container with 20 virgin females being added.

2. Field Studies

2.1 Working Area

Jozani forest, situated between 6°15'S - 6°16'S and 39°24' - 39°25'E, is the only remaining true primary forest on Unjuga island. The area comprises the forest reserve, covering approximately 5 km^2, with an important north-west extension towards Kisomanga, and in the north-east an extension towards Charawe. Previous surveys (2, 3) have shown that the forest harbours the highest densities of tsetse flies on the island.

An area of approximately 1 km^2 was chosen in the northern half of the forest (Figure 1) to conduct trial releases. Nine transects (N_1, N_2, etc.), each 1 km in length, were cut, starting in the open grassland at the western border of the forest and penetrating eastwards into dense thicket vegetation

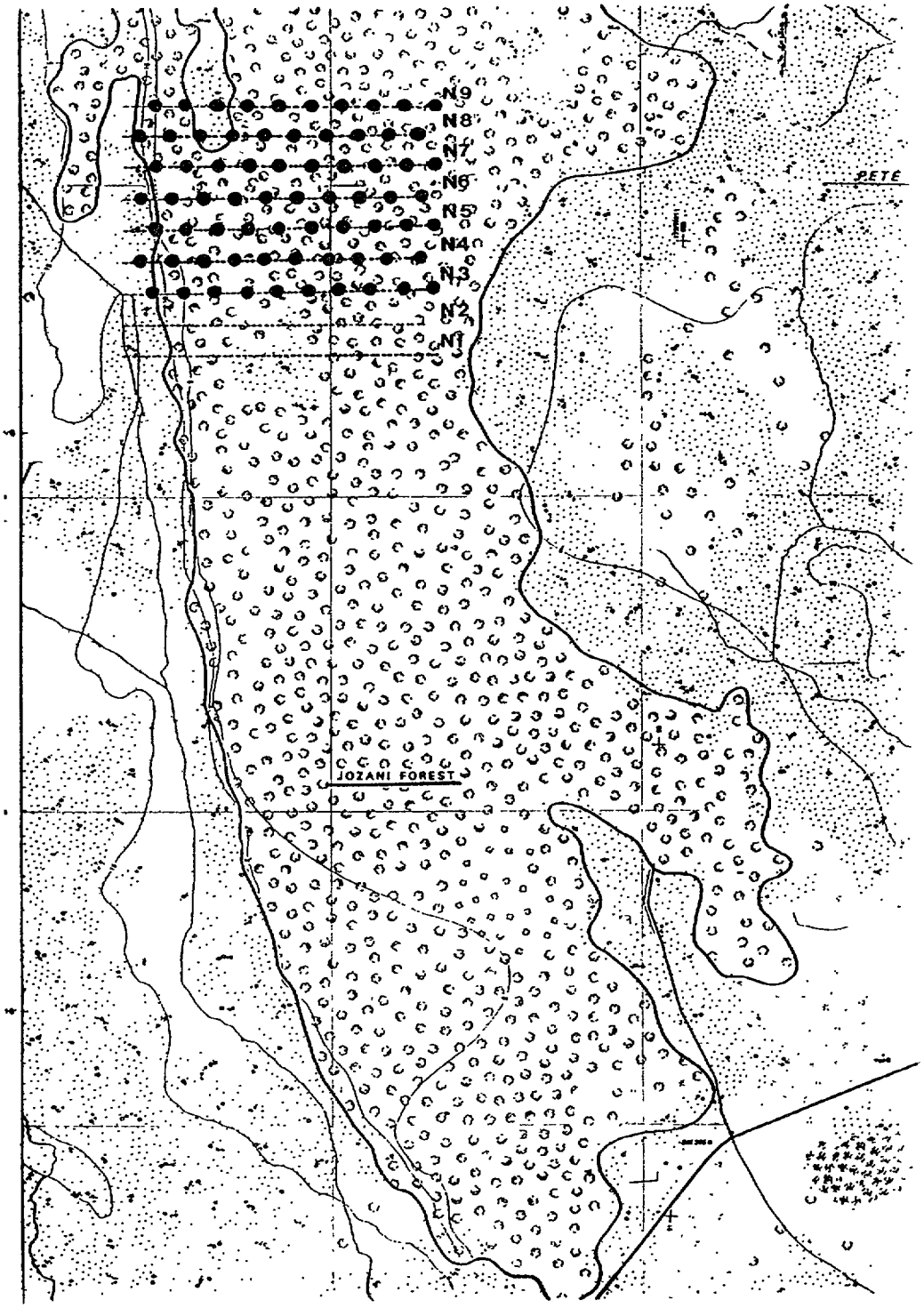

Fig. 1: An outline of the Jozani forest showing the experimental area with the nine (N1, N2....) transects. Each black dot represents a sticky panel.

of the forest edge before reaching the coral reef (a long north-south ridge of raised reefal limestone) after approximately 100 - 150 m. Immediately east of the ridge, a more open area, with sparsely scattered trees and a dense undergrowth of ferns and herbs, is encountered over a distance of 200 - 500 m. Towards the middle of the forest (last 400 - 500 m of the transects) there is more dense tropical rainforest.

2.2 Survey Techniques

Commonly used tsetse traps (Biconical, Modified biconical, Epsilon, F3) have proven to be completely ineffective in catching G. austeni on Unjuga island (2, 3, 4, 5). At present, the most appropriate devices for catching and monitoring tsetse populations are sticky panels (2, 4, 5, 6). Plywood, 4 mm thick, and fibreglass, were used to construct 56 Monopanels (MP) (5) and 14 3-Dimensional Targets (3-D) (6) respectively. Both types of sticky panels were painted with two layers of "Baby Blue" water resistant enamel paint and glued on both sides with Tanglefoot or isopolybuthylene. The panels were suspended with a rope and hung from an overhanging branch. This set-up enables free rotation of the target even with the slightest breeze.

For the pre-release survey, MP and 3-D were positioned at a density of 1 panel per 100 m of transect length in 7 of 9 transects. All panels were checked in the morning on a daily basis. Most of the females were dissected for determination of the ovarian age. For the males, the Wing Fray Category was recorded.

2.3 Pre-release Survey

(i) Catch distribution

Table 5 presents pooled data of fly distribution and average relative abundance (no. flies/trap/day) for 22 trapping days (from 2 November to 28 November 1990). Of the total 1060 flies caught during the pre-release period, 487 (45.9%) were females. The MP (n=50) caught 577 flies with 44.9% being females, whereas the 3-D (n=4) caugh 396 flies of which 47.9% were females. The relative abundance in the various transects is clearly not uniform. The apparent density is low in the thicket forest of the western forest edge and the more open area immediately east of the coral reef (panel I to V). The highest densities were in all transects found in the more dense vegetation (panels VI to X) towards the middle of the forest.

(ii) Age structure of flies

Table 6 shows the age structure of the 383 females which were dissected during the survey. During the first pre-release surveys (I), conducted from 8 November to 17 November 1990, 21.9% of the females were nulliparous and 46.5% were females with 1 or 2 ovulations. The proportion of females with 3 or more ovulations was 25.4%. A shift in age structure was observed during the second pre-release survey (II) (from 18 November to 28 November 1991). The proportion of nulliparous and females with 1 or 2 ovulation cycles increased to 23.0% and 59.1% respectively, while a decrease was observed for females with 3 or more ovulations (14.1%). The apparent natural abortion rate (i.e. parous females with empty uterus) was 5.3% and 2.6% for the two survey periods respectively.

Table 5. Catch distribution (relative abundance (no. flies/trap/day) at the different trap positions) of male and female G.austeni in transect N 3 - N 9 of Jozani North, during a pre-release survey of on average 22 trapping days

Transect No.	Trap site I		II		III		IV		V		VI		VII		VIII		IX		X	
	fem.	mal.	fem.	mal.	fem.	mal.	fem.	mal.	fem.	mal.	fem.	mal.	fem.	mal.	fem.	mal.	fem.	mal.	fem.	mal.
N 3	0.11	0.50	0.22	1.22	0.05	0.72	0.88	0.33	0.11	0.05	0.61	0.50	0.77	0.50	0.83	0.77	1.77	1.66	1.77	1.16
N 4	0.00	0.21	0.00	0.00	0.05	0.21	0.00	0.15	0.05	0.36	0.00	0.36	1.10	1.42	0.73	0.84	1.10	0.78	2.15	2.00
N 5	0.09	0.31	0.09	0.13	0.18	0.18	0.09	0.09	0.00	0.04	0.04	0.22	0.13	0.09	0.22	0.45	0.45	0.50	0.27	0.16
N 6	0.04	0.04	0.22	0.27	0.13	0.18	0.18	0.13	0.13	0.36	0.13	0.18	0.72	0.54	0.18	0.45	0.24	0.38	0.43	0.62
N 7	0.08	0.26	0.17	0.08	0.13	0.26	0.17	0.30	0.21	0.34	0.26	0.43	0.47	0.95	0.26	0.21	0.13	0.34	0.30	0.73
N 8	0.13	0.04	0.13	0.13	0.13	0.21	0.17	0.30	0.39	0.17	0.86	0.21	0.13	0.00	0.52	0.47	0.63	0.46	0.63	0.27
N 9	0.12	0.12	0.00	0.00	0.00	0.17	0.12	0.08	0.20	0.04	0.16	0.28	0.24	0.44	0.36	0.20	0.12	0.64	0.08	0.48

Table 6: Reproductive status of female G.austeni during 20 days of continuous pre-release trapping in Jozani north

Survey Period	No. of trapping days	No. of females	TENERAL	NULLIPAROUS	I AND II OVULATIONS						III AND MORE OVULATIONS					
					Normal egg	I and II instar	III instar	Degen. egg	Abortion	PL (2)	Normal egg	I and II instar	III instar	Degen. egg	Abortion	PL
			%	%	%	%	%	%	%	%	%	%	%	%	%	%
I (1)	10	114	0.9	21.9	18.4	27.2	0.0	0.0	3.5	0.9	6.1	18.4	0.0	0.0	1.8	0.9
II (1)	10	269	0.7	23.0	15.6	42.0	1.1	0.0	2.2	0.4	4.1	9.3	0.7	0.0	0.4	0.0

1. Pre-release survey I : from 8 Nov. till 17 Nov. 1990.
   Pre-release survey II: from 18 Nov. till 28 Nov. 1990.

2. Uterus empty due to post larviposition

The Wing Fray Category was determined for 523 males (91.2% of the total male catch). 90.8% and 84.1% of the males belonged to Wing Fray Category 1 or 2 for the two survey periods respectively, while only very few old males (category 4, 5 or 6) were caught (1.3% and 1.8%).

2.4 Transport of Sterile Males

(i) Transport conditions

All flies were packed in standard release cages at densities of 300, 500 or 600 to 800 males per cage and transported in wooden tranport boxes as described above. Only 10 minutes were required to transport the flies by Land Rover from TTRI to Tanga airport. There, the boxes were loaded into a small aircraft and flown (in 35-40 minutes time) to Zanzibar airport.

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

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

(ii) Survival rate of males during transport

During 10 trial releases, 22,563 sterile males were transported from Tanga to Jozani, and 20,699 (91.6%) were actually released (Table 7). It is clear that the fitness of the males transported under RS 1 was more affected (5.3% mortality plus 8.8% non-fliers) than under RS 2 (0.9% mortality plus 4.3% non-flyers).

The data presented in Table 8 corroborate the findings of the cage density tests carried out under laboratory conditions. In both release schedules, no differences in survival rate of sterile males after transport to the forest were found, irrespective of the density (300 to 800 flies/release cage).

2.5 Trial Release of Sterile female *G. austeni*

(i) Survival rate of sterile females during transport

Virgin females were given a 60 Gy gamma irradiation treatment on day 2-3 following emergence, and were individually colour-marked. Packaging and transport of sterile females was carried out as described for the sterile males under Release Schedule 1. In November 1990, 2,000 sterile females were transported to Zanzibar and 86.1% of them were actually released in transect N 7 of Jozani forest (Figure 1).

Table 7.: Survival records of sterile male G.austeni, transported from TTRI, Tanga to Zanzibar

No. males transported	Mortality		Alive Non flyers		Males released	
	No.	%	No.	%	No.	%
RELEASE SCHEDULE 1.						
7971	422	5.29	705	8.64	6844	85.8
RELEASE SCHEDULE 2.						
14592	136	0.93	631	4.32	13825	94.7
TOTAL						
22563	558	2.47	1336	5.92	20669	91.6

Table 8.: Survival records of Sterile Male G.austeni, transported at different release cage densities from TTRI, Tanga to Zanzibar

Male Density in Release Cage	No. males transported	Mortality		Alive Non-flyers		Males released	
		No.	%	No.	%	No.	%
RELEASE SCHEDULE 1.							
300	600	49	8.17	31	5.17	520	86.6
500	4485	221	4.93	416	9.28	3848	85.7
600 - 800	2866	152	5.27	258	8.94	2476	85.7
RELEASE SCHEDULE 2.							
300	2067	23	1.11	114	5.52	1930	93.3
500	4500	34	0.76	161	3.58	4305	95.8
600 - 800	8025	79	0.98	365	4.55	7581	94.4

Table 9.: Survival records of irradiated female G.austeni, transported from TTRI, Tanga to Zanzibar

No. females prepared in Tanga	Survivors DLDZ		Survivors Jozani		Females released	
	(a)	(b)	No.	%	No.	%
500 (*)	498	492	455	91.0	350	70.0
500 (*)	494	491	460	92.0	436	87.2
1000 (**)	999	989	988	98.8	936	93.6

(*) Released on 09.11.1990
(**) Released on 16.11.1990

(a) Evening
(b) Morning

(ii) Insemination status of females at recapture

All panels in the transects were checked daily and recovered sterile females were dissected to examine status and ovarian development.

Females recovered on day 1 post-release were not inseminated, but on day 2, 50% of them had already positive spermathecae. All sterile females recovered from day 5 on were inseminated, with the exception of one found on day 7.

The configuration in the reproductive system of recovered females (Table 10) confirmed the laboratory findings. In females recovered up to day 13 following treatment, development of the first two follicles was normal. Egg maturation was observed for the first and second follicle in ovulation sequence A, C. However, in older females, no development in B and D occurred and ovary atrophy followed.

Survival of the treated females in the natural habitat appeared to be very good, with some of them being caught 44, 57 and 68 days following release.

Table 10.: Recapture rate and reproductive status of sterile G.austeni females released in Jozani North. (1722 females released in 2 sessions)

Days following release	Females recaptured			Reproductive status					
				F.N.O.S.*			Ovaries Atrophied	Uterus content	
	Total	Non Inseminated	Inseminated	A	C	B		Egg	Empty
1	2	2	-	2	-	-	-	-	3
2	8	4	4	8	-	-	-	-	8
4	3	2	1	3	-	-	-	-	3
5	2	-	2	2	-	-	-	-	2
6	1	-	1	1	-	-	-	-	1
7	3	1	2	2	1	-	-	1	2
8	1	-	1	1	-	-	-	-	-
11	1	-	1	-	1	-	-	-	1
12	3	-	3	-	3	-	-	3	-
13	3	-	3	-	3	-	-	3	-
15	4	-	4	-	-	1	3	4	-
16	1	-	1	-	-	-	1	1	-
18	1	-	1	-	-	-	1	1	-
20	1	-	1	-	-	-	1	1	-
25	2	-	2	-	-	-	2	-	2
27	1	-	1	-	-	-	1	-	1
44	1	-	1	-	-	-	1	-	1
57	1	-	1	-	-	-	1	-	1
68	1	-	1	-	-	-	1	-	1
Total	40	9	31						

* F.N.O.S. = Follicle next in ovulation sequence.

Conclusion

The production of distributable excess by the G. austeni colony maintained at Tanga made it possible to initiate both laboratory and field testing of practical procedures for deployment of sterile flies.

Pre-release surveys in the northern part of Jozani forest, Zanzibar, confirmed the usefulness of sticky panels as monitoring device (see paper by Höreth-Böntgen, p. 185). The panels attracted adequate numbers of female flies (45% of the total catch), which is particularly important during a release programme when induced sterility is to be demonstrated. Survey data also clearly showed gradients in density of flies from the central part of the forest to the forest edges.

The recovery of inseminated radiation-treated female flies, a few days after and up to 68 days following their actual release in the natural habitat, also indicated the usefulness of this approach for monitoring the presence of native male flies and the success of control operations.

The release-recapture data for the more than 20,000 sterile males successfully transported from Tanga to the island (91% were actually released in Jozani forest as good flyers), indicated good adaptation, dispersal and survival.

The methods described in this report together with the experience gained during preliminary laboratory and field tests, will enable the project teams to use the SIT as an effective component of the ongoing efforts to control and eventually eradicate G. austeni from Zanzibar.

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TSETSE, TRYPANOSOMIASIS AND CATTLE RAISING IN SOUTHERN ANAMBRA STATE, NIGERIA

(Summary)

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Observations on flourishing resident herds and trade cattle within the Awka Zone in northern fringes of the rain forest belt of Anambra State, Nigeria, prompted this current study on the status of trypanosomiasis and tsetse distribution in the zone. Parts of the greater Mamu forest reserve and seven other forest patches covering 800 km^2 were studied. Except for two small forests others were infested with Glossina palpalis palpalis. 84 flies were captured, out of which one had mature Trypanosoma vivax infection. 443 cattle were examined in two slaughterhouses for trypanosomes. Only four of them had parasites that were identified morphologically as T. vivax. Deforestation due to industrialisation and development of the hinterland could contribute to lowered intensity of infection in an area originally thought very hazardous for livestock keeping.

COMPARISON OF BAITED AND UNBAITED NGU AND BICONICAL TRAPS FOR *Glossina pallidipes* AND *G. longipennis* AND THE CONTRIBUTION OF ODOUR COMBINATIONS IN NGURUMAN, KAJIADO DISTRICT, KENYA

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Abstract

Unbaited biconical, unbaited NGU, baited biconical and baited NGU traps were compared in a 4 x 4 Latin Square experimental design in three replicates. Acetone dispensed at 150 mg/h, cow urine (1000 mg/h) and 1-octen-3-ol (20 mg/day), i.e. the components of the odour-bait set in the tsetse suppression programme in Nguruman, were used. The contribution of the odours, both singly and in pairs, in catching two tsetse species, was also studied, with the complete odour set as control.

The unbaited NGU trap caught 1.74 - 2.5x more female flies than the unbaited biconical trap for G. pallidipes and G. longipennis, the increase being more pronounced for female catches. The index of increase was reduced slightly when both traps were baited. However, the biconical trap improved catches 8-11x when baited for both species. The NGU trap, when baited, increased the catches similarly.

Cow urine and acetone appeared to be the most effective odour pair for G. pallidipes, with the odours either acting synergistically or supplementarily. They contributed to over 90% of the total catch. The results were similar for G. longipennis, but at a lower contribution rate. Taken individually, cow urine was the dominant odour attractant for G. pallidipes, being responsible for nearly 60% of the catches. For G. longipennis, 1-octen-3-ol appeared dominant, but with a lower margin.

The NGU trap was more effective compared to the biconical trap, especially when the inexpensiveness and simplicity of the former is considered. Cow urine appeared to be the dominant attractant combining well with acetone for both G. pallidipes and G. longipennis.

Introduction

Odour attractants are playing an important role in trapping Glossina pallidipes and G. longipennis during survey and control activities. Acetone tends to increase G. pallidipes catches with increasing dose rates, while 1-octen-3-ol and a number of phenols are more attractive at lower doses (Dransfield, et al., 1986). The use of naturally derived animal odours dates back to the work of Vanderplank (1944), Langridge (1960), Park (1966) and Persoon (1966). They showed that animal extracts and skin scrapings attracted G. pallidipes and G. fuscipes. Chorley (1948) demonstrated that cattle dung and urine baited grass and plant branches attracted more tsetse than unbaited ones for several weeks. Hargrove (1978) and Owaga (1985) further showed that ox-odour and buffalo urine respectively increased G. pallidipes catches 6 and 10 fold. Acetone and 1-octen-3-ol have been isolated from ox-breath (Hall, 1985). Seven phenolic compounds, including phenol, 3- and 4-n-propylphenols,

have been isolated from cow and buffalo urine (Hassanali et al., 1986). Vale and Hall (1985) also noted ketones, aldehydes and fatty acids as potential tsetse attractants. This was confirmed by Saini and Dransfield (1987).

The tsetse control trial at Nguruman, based on odour-bait trapping technology (Dransfield et al., 1991), relies on the use of acetone dispensed at 150 mg/h, cow urine at 1000 mg/h and 1-octen-3-ol at 20 mg/day.

The work reported analyses the contribution of odours used singly, in pairs or as total odour set compared with NGU 2G (Brightwell et al., 1991) and biconical trap catches, using the total odour set as control.

Materials and Methods

Unbaited biconical, unbaited NGU 2G, baited biconical and baited NGU 2G traps were compared in a 4 x 4 Latin Square design (replicated three times) with unbaited biconical traps as control. The effectiveness of individual and combined odours was tested using a NGU 2G trap. The total odour set was used as control. Acetone, cow urine and 1-octen-3-ol were dispensed simultaneously as described by Dransfield et al. (1990). Acetone was dispensed at the rate of approximately 150 mg/h from 200 ml bottles fitted with a metal lid with a 2 mm hole. The cow urine dispenser consisted of 5 ml 1-octen-3-ol, completely sealed in a 3 x 3 cm tetrapak made from plastic tubing. The release rate through its total surface was about 20 mg/day. The 2 l plastic container with cow urine had a 2 x 4 cm opening cut in the middle of the upper surface through which 1-octen-3-ol was also dispensed. The sets were placed on the ground, at 30 cm from the trap.

Acetone, cow urine and 1-octen-3-ol were compared separately in a 4 x 4 Latin Square experimental design (replicated three times) using NGU 2G traps with the total odour set as control. The three odours were also compared in pairs with the total odour set as control. The experiments were carried out in the same experimental blocks. Twelve traps, at least 200 m apart, were set at night, and the odours and cages were introduced the following morning at 06.15 h. The traps were emptied daily at 06.15 h, and at the same time odour positions were changed according to the experimental design. Flies in the catches were identified, sexed, counted and recorded. The data for males and females of each species were recorded separately and subjected to analysis of variance after a log (x+1) transformation.

Results

Data in Table IIa show that the complete odour set caught significantly more ($p<0.001$, $r^2 = 0.97$) male G. pallidipes than each of the three odour components dispensed separately. However, cow urine contributed 42% of the total catches which was significanlty higher than individual catches for acetone or 1-octen-3-ol alone. This pattern of response was also pronounced in female G. pallidipes catches ($p<0.01$, $r^2 = 0.89$) where the contribution of cow urine increased to 58%.

In the case of male G. longipennis (Table IIb), the complete odour set attracted significantly more ($p<0.01$, $r^2 = 80$) flies than cow urine or acetone. There was, however, no significant difference between the complete odour set catch and that of 1-octen-3-ol which, in turn, attracted significantly more male G. longipennis (54%) than did acetone (27%). Whereas 1-octen-3-ol attracted more female G. longipennis (38%) than did acetone and cow urine, each of the three odours attracted significantly less flies than the complete odour set.

Table I. Effect of odours on NGU and biconical trap catches

a) Response of G. pallidipes to baited and unbaited biconical and NGU traps

	Males			Females		
	Detrans Mean	Index of Increase	Percentage in Sample	Detrans Mean	Index of Increase	Percentage in Sample
Unbaited biconical Trap	9.94	1.00	51.66	9.30	1.00	48.34
Unbaited NGU trap	17.33	1.74	42.20	23.74	2.55	57.80
Baited biconical trap	116.39	11.71	53.59	100.80	10.84	46.41
Baited NGU	143.18	14.40	47.51	158.32	17.02	52.49

b) Response of G. longipennis to baited and unbaited biconical and NGU traps

	Males			Females		
	Detrans Mean	Index of Increase	Percentage in Sample	Detrans Mean	Index of Increase	Percentage in Sample
Unbaited biconical Trap	0.27	1.00	69.23	0.12	1.00	30.77
Unbaited NGU trap	0.30	1.11	53.57	0.26	2.12	46.43
Baited biconical trap	3.84	14.12	79.50	0.90	8.08	20.50
Baited NGU	2.31	8.48	51.68	2.16	17.68	48.32

Table II.

a) Effect of acetone, cow urine and 1-octen-3-ol combination on G. pallidipes catches

	Males			Females		
	Detrans Mean	Index of Increase	Percentage in Sample	Detrans Mean	Index of Increase	Percentage in Sample
1-octen-3-ol/cow urine/ acetone (control)	59.86	1.00	41.85	83.18	1.00	58.15
Acetone	15.61	0.26	34.82	29.23	0.35	65.18
Cow urine	25.20	0.42	34.19	48.52	0.58	65.81
1-octen-3-ol/acetone	12.25	0.20	33.44	24.38	0.29	66.56

b) Effect of acetone, cow urine and 1-octen-3-ol combination on G. longipennis catches

	Males			Females		
	Detrans Mean	Index of Increase	Percentage in Sample	Detrans Mean	Index of Increase	Percentage in Sample
1-octen-3-ol/cow urine/ acetone (control)	2.04	1.00	45.83	2.42	1.00	54.17
Acetone	0.55	0.27	43.52	0.71	1.30	56.48
Cow urine	0.19	0.09	26.44	0.53	0.22	73.56
1-octen-3-ol	1.10	0.54	54.74	0.91	0.38	45.26

There was no significant difference ($p<0.01$, $r^2 = 0.94$) between male G. pallidipes catches for the traps with total odour set or cow urine and acetone dispensed simultaneously. The latter attracted 84% of the total catch (Table IIIa). The traps with 1-octen-3-ol/cow urine and 1-octen-3-ol/acetone combinations caught significantly less than the ones with both the total odour set and cow urine/acetone combination. The cow urine/1-octen-3-ol combination yielded the lowest catches. The pattern of response for female G. pallidipes to the paired odour combinations was similar to that of the males ($p<0.001$, $r^2 = 0.94$). The cow urine/acetone combination were responsible for 92% of the total odour set catches, again with the cow urine/1-octen-3-ol combination providing the lowest catches.

Table III. Effect of odour combination on tsetse catches

a) Effect of acetone, cow urine adn 1-octen-3-ol combination of G. pallidipes

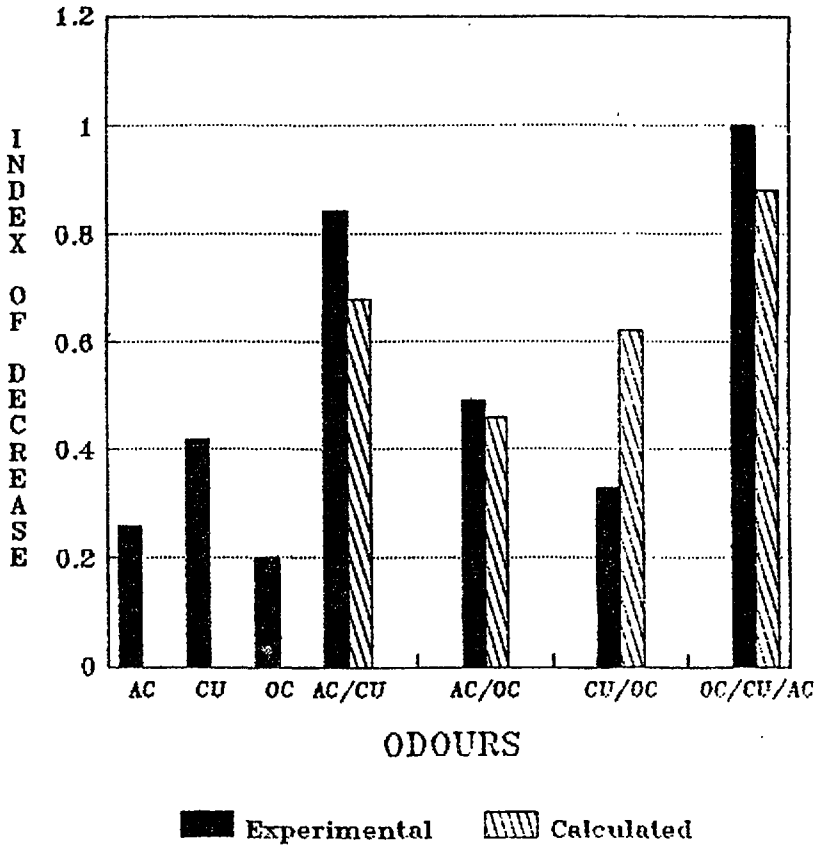
	Males			Females		
	Detrans Mean	Index of Increase	Percentage in Sample	Detrans Mean	Index of Increase	Percentage in Sample
1-octen-3-ol cow urine/ acetone (control)	59.03	1.00	27.18	158.14	1.00	72.82
1-octen-3-ol/cow urine	19.30	0.33	24.46	59.61	0.38	75.54
Cow urine/acetone	49.57	0.84	25.42	145.43	0.92	74.38
1-octen-3-ol/acetone	29.09	0.49	28.20	74.05	0.47	71.80

b) Effect of acetone, cow urine and 1-octen-3-ol combination on G. longipennis catches

	Males			Females		
	Detrans Mean	Index of Increase	Percentage in Sample	Detrans Mean	Index of Increase	Percentage in Sample
1-octen-3-ol/cow urine/ acetone (control)	1.64	1.00	31.84	3.51	1.00	68.16
1-octen-3-ol/cow urine	0.33	0.20	46.48	0.38	0.11	53.52
Cow urine/acetone	0.94	0.57	31.54	2.04	0.58	68.46
1-octen-3-ol/acetone	1.06	0.64	45.49	1.27	0.36	54.51

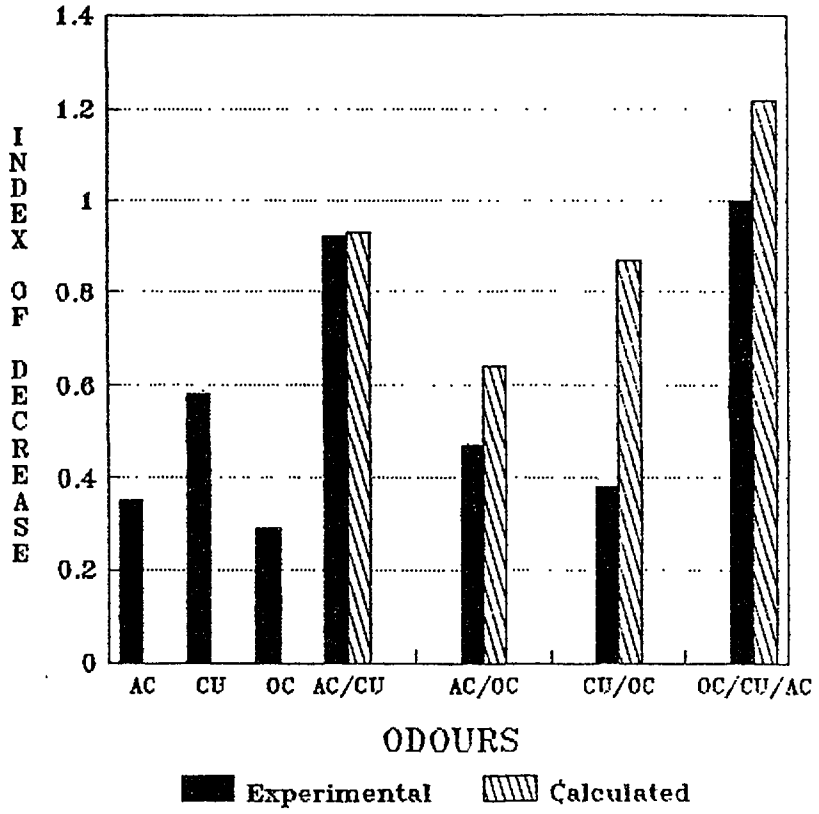
For male G. longipennis there was no significant difference ($p<0.001$, $r^2 = 0.79$) between acetone/cow urine and acetone/1-octen-3-ol combinations and the complete odour set. The paired odours contributed 57% and 64% respectively, of the total catch (Table IIIb). Cow urine/1-octen-3-ol attracted significantly less male flies than the total odour set. The three odour pairs attracted significantly less ($p<0.0001$, $r^2 - 0.93$) female G. longipennis than the total odour set. The acetone/cow urine combination was the most effective of the odour pairs, accounting for 58% of the total catch. The contribution of acetone/1-octen-3-ol (36%) was also significantly higher ($p<0.05$) than that of cow urine/1-octen-3-ol.

Figures 1-4 show the individual contribution of acetone, cow urine and 1-octen-3-ol and the three odour pairs to G. pallidipes and G. longipennis catches as a proportion of the complete odour set. They compare the calculated arithmetic values of indices for increase of individual odours with that of the paired and complete odour set. When acetone was dispensed simultaneously with cow urine, there was a synergistic effect which was more pronounced in males of both tsetse species than in females. For the acetone and 1-octen-3-ol pair, the values for the jointly despensed odours were lower than the calculated ones, but still higher than their contributions taken individually. This trend was not observed for the cow urine/1-octen-3-ol pair, for which values lower than the calculated ones were found. Lower values indicate repellency for either one or both of the odours.



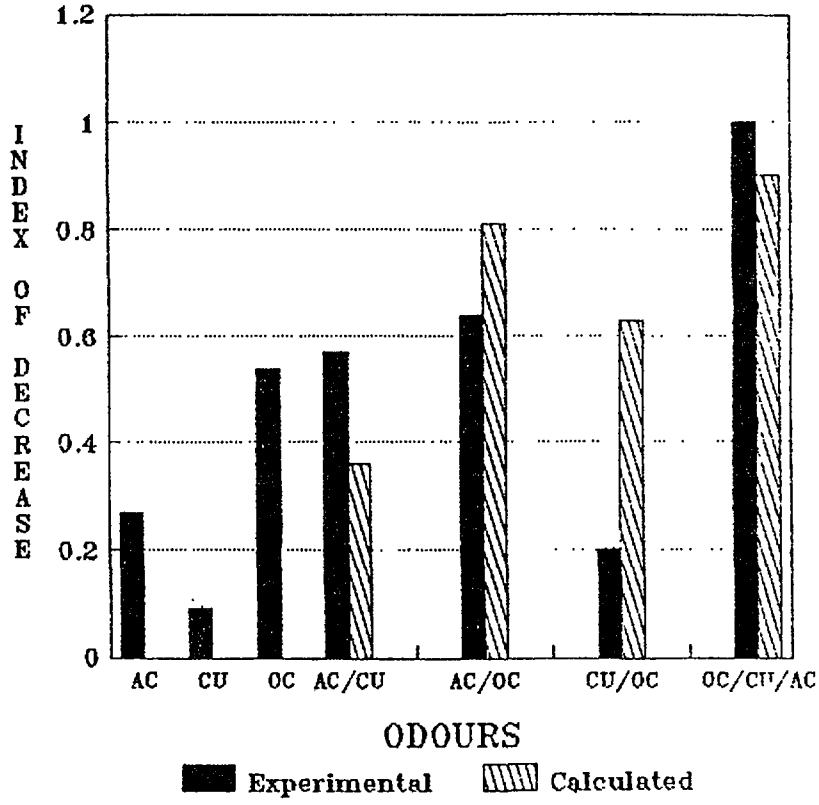
AC = acetone, CU = cow urine, OC = octenol

Figure 1. Influence of Odours on Tsetse catches in Nguruman; male G. pallidipes



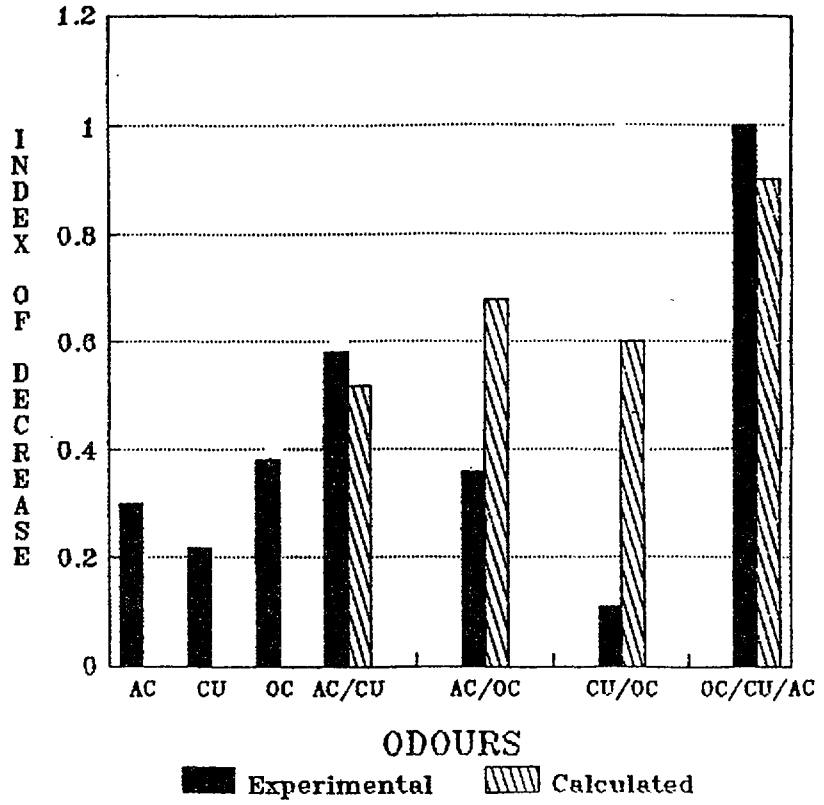
AC = acetone, CU = cow urine, OC = octenol

Figure 2. Influence of Odours on Tsetse catches in Nguruman; female G. pallidipes



AC = acetone, CU = cow urine, OC = octenol

Figure 3. Influence of Odours on Tsetse catches in Nguruman; male G. longipennis



AC = acetone, CU = cow urine, OC = octenol

Figure 4. Influence of Odours on Tsetse catches in Nguruman; female G. longipennis

Discussion and Conclusions

Unbaited NGU traps caught significantly more G. pallidipes males (r^2 = 0.95, $p<0.001$) and females than unbaited biconical traps (Table Ia). The increase was 1.74x for males and 2.55x for females, making the baited trap suitable for controlling G. pallidipes (Brightwell et al., in press). For G. longipennis, there was no significant difference between the unbaited and baited traps (r^2 = 0.82, $p<0.001$).

A number of active tsetse odour attractants have been identified from ox-breath, including carbon dioxide, acetone and 1-octen-3-ol (Vale, 1980; Bursell, 1984; Vale and Hall, 1985). Of these, carbon dioxide has not been dispensed conveniently for use under field conditions. Buffalo urine has been shown to contain phenol, 3- and 4-cresols, 3- and 4-ethylphenols and 3- and 4-n-propylphenols (Hassanali et al. 1985). In addition to ketones, aldehydes and fatty acid deserve further investigation as additional attractants (Vale and Hall, 1985). Cow urine has also been shown to contain attractants which are not significantly different from buffalo urine (Dransfield et al., 1986) Although in nature, these attractants are generated simultaneously from animals, their use with tsetse traps have resulted in considerable increase in catches compared to unbaited traps (Table I). Although in each treatment, the geometrical means for the G. pallidipes is lower than for G. longipennis, the indices of increase for male (11.71x) and female (10.84x) were generally similar to those of male (14.12x) and female (8.08x) G. longipennis for the biconical traps. It appears that due to the initially higher catches with the unbaited NDU 2G trap, the indices of increase when the trap was baited were lower than for the biconical trap both in G. pallidipes (male - 8.26x, female - 6.67x) and G. longipennis (male - 7.67x, female - 8.33x). The two species responded similarly to the odour attractants despite the lower

geometrical means for G. longipennis for both traps. The difference in the trap catches for this species is apparently due to either its relatively low density or its poor response to the two trap types.

In the case of G. pallidipes, the difference between catches of the two traps decreased slightly when the odour baits were used. Thus, the difference between NGU 2G and biconical traps was reduced from 1.74x and 2.55x for males and females, respectively, to 1.23x and 1.57x when traps were baited. This decrease might be explained by a concentration of flies around the trap under the influence of the odour set.

Compared with the total odour set, the three odours, when individually used, attracted significantly less flies of both sexes of G. pallidipes (Table IIa). However, cow urine significantly increased the catch (r^2= 0.97, $p<0.001$) for males and r^2 = 0.89, $p<0.01$ for females) compared to both acetone and 1-octen-3-ol, attracting 42% and 58% of the total catch for males and females, respectively. In the case of the G. longipennis, cow urine attracted only 9% of the males and 22% of the females, whereas this species responded positively to 1-octen-3-ol (54% for males, 38% for females) with no significant difference between the male catches for the total odour set. The response of G. pallidipes and G. longipennis to acetone was similar.

The most attractive odour pair, acetone/cow urine, attracted 84% of the males and 92% of the females of G. pallidipes (Table III). There was no significant difference between the pair and the total odour set (r^2 = 0.94, $p<0.001$ for males, r^2 = 0.94, $p<0.001$ for females). The trend was similar for G. longipennis where it contributed 57% and 58% for males and females, respectively. Dransfield et al. (1986) reported these dramatic increases for biconical traps with various dose rates. The acetone/1-octen-3-ol combination caused a rather suppressed response in both species except in male G. longipennis (r^2 = 0.79, $p<0.001$), for which the pair contributed 64% of the total catch. The cow urine/1-octen-3-ol pair had the lowest response from both species.

When the contribution of odours dispensed individually or jointly is examined for G. pallidipes (Figures 1-2), a synergistic effect on the males is noted for the acetone/cow urine pair. For females, this effect appears to be supplementary. Acetone/1-octen-3-ol had a suppressed synergistic effect while cow uringe/1-3-ol combination appeared to express repellency. For G. longipennis (Figures 3-4), synergism was expressed in the acetone/cow urine pair by both sexes. However, as in the case for G. pallidipes, the cow urine/1-octen-3-ol pair showed repellency.

The three odours gave the highest catches when dispensed simultaneously. However, for G. pallidipes, the acetone/cow urine combination gave satisfactory catches. Thus, in control programmes they could be used without addition of 1-octen-3-ol. Cow urine could be used alone where there are difficulties in acquiring acetone. For G. longipennis, it appears that all three odours should be dispensed simultaneously, with 1-octen-3-ol being a very essential component.

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ACTIVITIES OF THE TSETSE CONTROL UNIT, BOTSWANA
(Summary)

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Introduction

Botswana is at the extreme southern extent of the African tsetse belt, and most of the country does not provide a suitable environment for the maintenance of breeding tsetse fly populations. The principal permanent habitat is the Okavango Delta and its associated northern river system. The Okavango Delta is a sparsely settled area of permanent swamp, seasonal floodplain and low-lying islands. Tsetse existed in the delta long before written history of the area came into existance. Much of this infestation died out during the great rinderpest pandemic at the turn of the century, which decimated the host animals. Following collapse of the fly population, people took advantage of the situation and occupied more hospitable parts of the delta. However, the fly population eventually expanded to meet the settlers and in the early 1940's a conflict of interest arose. The Department of Tsetse Fly Control was subsequently formed.

Tsetse Fly Control

Early methods of tsetse control included bush clearing and game animal destruction. These have long been abandoned since they are both ineffective and environmentally unacceptable.

Traps and Targets

Much publicity has been given recently on the use of odour-baited traps and chemically impregnated targets as an alternative and environmentally more acceptable form of control. The method is unfortunately both ineffective and impractical in this country for two reasons: (i) The only species of tsetse found in Botswana is *G. morsitans*, which, being a more open country "Savannah" species appears to rely more on sight and movement than on odour attractants and is much less liable to enter traps than the riverine species. (ii) The logistics of deploying large numbers of traps and targets when covering wide areas is always formidable, in the terrain found here, it is virtually impossible.

Because of these factors, Botswana has relied almost exclusively on aerial application of non-persistant insecticides and has done a great deal of work in understanding and refining the technique.

Aerial Spraying

Aerial spraying trials were begun in 1972/73 and the first large scale application was in 1974. Eradication following aerial application was not achieved until 1977 when 3000 km^2 of the southeastern section of the delta were successfully cleared. Due to limited funds and the extensiveness of the fly belt, eradication has not been possible.

Instead, we are engaged in a programme of integrated large scale annual spray campaigns in an attempt to obtain a major reduction in the fly. Areas sprayed in the subsequent years are as shown in table 1.

Table 1.

Fixed - wing sequential aerial spraying - Botswana

Year	1973	1974	1975	1976	1977	1978	1979	1980
km^2 area sprayed	1500	1300	2700	2500	4000	2500	3000	1800

Year	1981	1982	1983*	1985*	1986*	1987*	1989*	1990*
km^2 area sprayed	5000	6000	9500	6000	5800	4000	6800	5500

* All operations used Endosulfan except from 1983-1990 when a cocktail of Endosulfan/Pyrethroid was used.

Summary and Conclusion

1. All sections of the delta have been sprayed more than once.

2. There are very few flies left in the delta proper - only isolated pockets.

3. No cases of Tryps have been detected in cattle surrounding this area for several years. Over 100,000 head of beef cattle graze in places which would, in the absence of tsetse control, be slightly or heavily infested with flies.

4. A solid fly belt still exists in the northern areas of the Kwando/Linyanti complex, which extends into Zambia, Angola and Namibia.

5. It will never be possible to maintain the delta fly free as reinvasion from the above areas is a constant threat.

6. The presence of the fly threatens beef production, which is one of the country's economic backbone.

7. Continued control by the current technique is expensive. All programmes are funded from the national budget.

8. A "one off" eradication programme makes the most practical and economic sense. The operation should be a joint regional one covering the entire belt.

ANIMAL TRYPANOSOMIASIS CONTROL IN UGANDA

(Summary)

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Geographically, Uganda is situated between longitudes 35° 03' E and latitudes 4° 15' 1° 30' S. She covers an area of 240,000 km^2 and 192,000 km^2 is land and the rest water. She is at an altitude of 400-1500 m and enjoys a cool climate of 15-25°C with an average annual rainfall of 900-1500 mm. This type of climate favours a natural vegetation of equatorial forests to Savanna grasslands with a good network of permanent rivers.

Uganda is in the hinterland bordering with the Republics of Kenya (east), Sudan (north), Tanzania and Rwanda (south) and Zaire (west).

Uganda, with a population of about 17 million people, depends largely on the Agricultural Sector both for subsistance and export. Uganda livestock stands at 4 million cattle, 2.5 million goats, 0.7 million sheep and 0.5 million pigs. The life of these animals is being threatened by various tropical diseases. The most common ones in Uganda are tickborne diseases, trypanosomiasis, rinderpest, CBPP, FMD, etc.

For the sake of this paper, I will dwell mainly on animal trypanosomiasis control with particular emphasis on protecting the national herd.

Animal trypanosomiasis is endemic in all the 34 districts of Uganda except the highland areas of the country. These are correspondingly areas of high tsetse fly infestation. For effective control of this disease, therefore, an integrated approach is adopted.

The country has been divided into three zones of disease incidence, namely the high, medium and low risk zones. Each zone has a slightly different treatment regime with curative and prophylactic trypanocidals as short term control measures. The long term aim is to eradicate the tsetse fly, and therefore, the disease.

Field problems

Uganda, being one of the developing countries, the control measures has been affected by certain constraints:

(i) Poor laboratory facilities in the field.
(ii) Inadequate motivation of extension staff.
(iii) Lack of reliable transport.
(iv) Shortage of both curative and prophylactic trypanocides.
(v) Lack of sufficient funds to run the programme.

Recommendation

Some of the recommendations are aimed at improving on the above mentioned constraints.

TSETSE AND TRYPANOSOMIASIS CONTROL FOR THE RURAL DEVELOPMENT OF BUVUMA ISLAND, LAKE VICTORIA, UGANDA

(Summary)

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Historically, the potentially highly agricultural productive areas of Uganda, bordering the shores of Lake Victoria, have been plagued by the presence of tsetse flies, which has limited the development of the livestock industry through the transmission of animal trypanosomiasis and devastated human populations due to sleeping sickness epidemics. The Busoga region has been one of the heavily affected areas and include the Buvuma islands located in the northern part of the lake.

While Glossina pallidipes was, in the past, reported to be the main vector in the area, evidence has accrued in recent times which indicates that G. fuscipes fuscipes is in fact the main vector on the island. There is also evidence that the species has adopted a peridomestic behaviour in this, predominantly, farming and fishing community. An increase in human and animal population has been ovserved, partly due to emigration from the mainland area of Busoga but also because Buvuma Island offers fallow land and natural pasture attractive to settlers.

Since 1987/88, intensive surveys were conducted and current monitoring work concentrates on strategic sites such as water collection points, fishing camps, ports of call and selected villages on the island (Bulopa-Walwanda, Lwenyanga, Kyanamu, Tome, Isiriba and Kachanga). In addition, regular trapping is carried out at Kiyindi picket on the mainland in order to catch flies that are carried across by paddle canoes and motorboats. Field base camps for survey and control teams were established at Katamiro and Magyo.

Making use of 20-25 pyramidal traps impregnated with deltamethrin (400 mg/trap) for 20-22 days/month, the G. f. fuscipes population around reference villages was considerably reduced (90-95% after 7-9 months).

Arrangements are underway to evaluate insecticide treatments of domestic livestock host animals and monitor trends in the transmission of trypanosomiasis. In collaboration with UTRO, Tororo, experimental rearing of G. f. fuscipes will be undertaken aiming at producing flies for mark-release-recapture studies and trials with sterile flies.

TSETSE AND TRYPANOSOMIASIS AT THE NGULIA RHINO SANCTUARY

(Summary)

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The conservation of black rhinoceros (Diceros bicornis) in Kenya involves the translocation of rhinoceros to protected sanctuaries, e.g. the recently enlarged 69 km^2 sanctuary at Ngulia in Tsavo West National Park. Management of animal health during translocations, especially in terms of trypanosomiasis, has yet to be studied in detail, largely because of the urgency of protecting animals from being poached. As the black rhinoceros is known to be particularly susceptible to infection with Trypanosoma brucei, there is a strong possibility that the stress of translocation will induce health problems that would not normally occur in resident populations. Similarly, the movement of animals born in tsetse-free areas to tsetse-infested areas may result in trypanosomiasis problems because of loss of immune protection in the absence of challenge in early life. Finally, there is some concern over the possibility of facilitating genetic exchange in T. brucei through the artificial movement of animals over long distances into novel environments.

To aid in the development of prudent management practices, we initiated baseline studies on tsetse distribution and trypanosomiasis at the Ngulia sanctuary. To date, we have completed a dry season survey of tsetse distribution over a 125 km^2 area centred on the sanctuary and have characterized the nature of the trypanosomiasis challenge in different areas. Parasites from tsetse have also been isolated and are being characterized with DNA probes and other techniques. Trypanosomiasis challenge at Ngulia involves a variety of Trypanosoma spp., as would be expected from any wildlife area. Nevertheless, an unusual finding is the lack of diversity in Nannomonas parasites, with most belonging to the savannah group of T. congolense.

So far, one experimental rhinoceros has been moved to the sanctuary. This individual succumbed to a cryptic T. congolense infection that could not be detected with normal parasitological techniques. However, we were successful in detecting the infection through xenodiagnosis on two occasions. The animal lost condition following translocation, possibly for nutritional reasons, but it eventually recovered. Future work will concentrate on various aspects of the susceptibility of rhinoceros to trypanosomes, and the use of trypanocides to manage infections during translocations.

AN EVALUATION OF THE SUPPRESSION OF *Glossina pallidipes* BY THE ODOUR-BAITED INSECTICIDE-COATED TARGETS IN THE LAMBWE VALLEY, KENYA

(Summary)

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From August 1988, odour-baited insecticide-coated targets have been placed and maintained every three months in Ruma National Park, Lambwe Valley, western Kenya, to eliminate G. pallidipes from the area. The extent of tsetse suppression was assessed from estimates of absolute and apparent densities, age grades and insemination performance of monthly samples using odour-baited biconical traps from January-December 1990. On the basis of data obtained before targets had been deployed, the monthly reduction rates in absolute and apparent densities varied between 96.5-99%, respectively. The highest reductions occurred consistently during the first month following each target servicing cycle after which flies recovered gradually until the next operation. Age grading showed that females and males of all age groups were present throughout. The recapture of marked individuals of either sex at or over three months old indicated that flies could mate, breed and survive over two successive target servicing occasions. There was no evidence of break-down in males/females encounters by targets as samples of non-teneral females were all adequately inseminated. Results showed that probably hundreds of thousands of satisfactorily reproducing flies still existed in Ruma National Park. Thus, although a relatively high level of suppression of G. pallidipes was attained, eradication is still far from being achieved.

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