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THE INTERNATIONAL CENTRE OF INSECT PHYSIOLOGY AND ECOLOGY

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Eighth Annual Report 1980

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THE INTERNATIONAL CENTRE OF INSECT PHYSIOLOGY AND ECOLOGY

EIGHTH ANNUAL REPORT

1980

Nairobi, October 1981

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Key to Annotations

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**	Member of the Programme Committee
***	Member of the Nominating Committee
I	First 3-year tenure
II	Second (and final) 3-year tenure
C	Elected by the ICIPE Company
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Deputy Director (Research), Professor A.S. Tahori
Financial Manager, Mr. L.Z. Mosha
Manager for Communication Systems, Mr. J.M. Ojal

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Mr. E.O. Omolo, Agronomist
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Mr. E.O. Arigi, Technician
Mr. S.O. Obiero, Technician
Mr. S.K. Oketch, Technician
Mr. F.O. Onyango, Technician
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Mr. P.E. Njoroge, Junior Technician
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Miss A. Ragot, Technical Assistant
Mr. P. Otieno, Subordinate Assistant

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Mr. A.G.L. Delobel, Research Scientist
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Mr. J.C. Olela, Senior Technician
Mr. P. Ollimo, Technician
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Mr. G.O. Amala, Technical Assistant
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Mr. G.E. Oloo, Subordinate Assistant

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Dr. J.A. Odebiyi, Visiting Research Associate

Arrivals

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Mr. J.J. Njoka, Agronomist

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Mr. R. Okello, Technical Assistant
Mr. G.M. Kinyanjui, Technical Assistant; Driver
Mr. G.N. Mburu, Subordinate Assistant

Departures

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Dr. B.I.P. Persson, Research Scientist

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Dr. G.W. Oloo, Research Scientist
Dr. J.P.E.C. Darlington, Research Scientist
Dr. B.M. Okot-Kotber, Research Scientist
Dr. R. Sieber, Postdoctoral Research Fellow
Dr. T.O. Oloya, Postdoctoral Research Fellow
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Mrs. M.N. Baraza, Technician
Mr. M.O. Kotengo, Junior Technician

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Mr. E. Nyandat, Technical Assistant
Miss M.G. Wanjiru, Technical Assistant

Departures

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Mr. D.K. Punyua, Associate Scientific Officer
Dr. G.M. Binta, Associate Scientific Officer
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Mr. F.T. Thuo, Technical Assistant
Mr. G.M. Hindi, Subordinate Assistant
Mr. J.G. Mugane, Subordinate Assistant
Mr. J.N. Ndungu, Subordinate Assistant
Mr. K.C. Wainaina, Subordinate Assistant

6. Tsetse Research Programme

Dr. A. Challier, Programme Leader

Tsetse Reproductive Physiology Project

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Dr. M.S. Ramasamy, Research Scientist
Mrs. R.W. Kunyiha, Research Assistant
Mr. F. Mukunza, Junior Technician
Mr. P.A. Osula, Junior Technician

Tsetse Ecology and Epidemiology Project

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Dr. D.A. Turner, Research Scientist
Mrs. M.L.A. Owaga, Scientific Officer
Miss S.A. Tarimo, Graduate Research Scholar
Mr. J.M. Apale, Technician
Mr. R. Mutuaruhiu, Junior Technician
Mr. D. Uvyu, Junior Technician
Mr. J.A. Makau, Technical Assistant
Mr. J.M. Muchiri, Technical Assistant/Driver
Mr. D.K. Mungai, Technical Assistant/Driver
Mr. J.K. Kiilu, Subordinate Assistant

Trypanosome – Vector Physiology

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Dr. T.K. Golder, Research Scientist
Dr. M.B.A. Nyindo, Research Scientist
Dr. C. Powell, Research Scientist
Miss N.F. Darji, Senior Research Assistant
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Dr. F.W. Mosha, Research Scientist
Dr. W.A. Otieno, Research Scientist
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Dr. J.B. Kaddu, Postdoctoral Research Fellow
Dr. M.O. Odindo, Postdoctoral Research Fellow
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Miss L.M. Oketch, Graduate Research Scholar
Dr. J.O. Olobo, Graduate Research Scholar
Miss D.M. Sabwa, Graduate Research Scholar
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Mr. P.M. Mwamisi, Junior Technician
Mr. M.C. Ngumah, Junior Technician
Mr. R.C. Odhiambo, Junior Technician
Mr. J.A. Otieno, Junior Technician
Mr. P. Amutalla, Technical Assistant
Mr. E. Mkuzi, Technical Assistant
Mr J. Mwandandu, Technical Assistant/Driver

RESEARCH UNITS STAFF

1. Chemistry and Biochemistry Research Unit

Dr. D.L. Whitehead, Senior Research Scientist
Dr. P.G. McDowell, Postdoctoral Research Fellow
Dr. D.A. Otieno, Postdoctoral Research Fellow
Mrs. M. Vundla, Associate Scientific Officer
Mr. A. Chapya, Chief Technician
Mr. N. Juma, Junior Technician

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Mr. B.N. Odero, Principal Technician
Mr. E.B. Ole Sitayo, Senior Technician
Mr. G. Achieng, Technical Assistant
Mr. L. Moreka, Technical Assistant

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Dr. L.R.S. Awiti, Postdoctoral Research Fellow
Mrs. J.A. Kongoro, Research Assistant
Mr. M. Chimtawi, Principal Technician
Mr. P. Lisamula, Senior Technician
Mrs. J. Murithi, Senior Technician
Mr. N. Ogoma, Junior Technician

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Mr. J. Ongudha, Junior Technician
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Mr. G.M. Birir, Technical Assistant/Driver

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Mr. J.B. Omulo, Junior Technician
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Mr. A. Muchiri, Driver
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Mr. J.N. Musisi, Maintenance Engineer
Mr. J. Atiche, Security Officer
Mr. S. Akhaya, Assistant Janitor
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Mr. J. Elegwa, Assistant Gardener

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Miss M. Wafula, Secretary
Miss R.J. Chesum, Assistant Secretary
Mr. A.O. Were, Messenger
Mr. D. Odhiambo Aoko, Messenger

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Mrs. R.P. Ortega, Documentalist
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Mr. M.P. Macointo, Supplies Assistant

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Mr. E. Omolo, Agronomist
Mr. B.S.K. Masyanza, Farm Controller
Mr. J.O. Angado, Maintenance Engineer
Mr. F.K. Onyola, Assistant Accountant
Mrs. M.N. Olach, Assistant Secretary
Mr. P. Mbuya, Technical Assistant/Driver

MBITA POINT INTERNATIONAL SCHOOL

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Mr. J.W. Weruinga, Teacher

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Mr. P.O. Nguri, Senior Accounts Clerk
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Mr. E.M. Sowah, Technical Assistant/Driver

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Miss Lucy Irungu, Graduate Research Scholar
Mr. J.I. Jondiko, Postdoctoral Research Scholar (at Nottingham University, U.K. since September 1980)

Dr. G.P. Kaaya, Postdoctoral Research Fellow
Mr. J. Kawooya, Associate Scientific Officer (at University of Illinois, Urbana since September 1977)
Mr. M. Kilu, Graduate Trainee
Mr. A. Mongi, Graduate Trainee
Mr. P. Njagi, Graduate Trainee
Mr. J.J. Njoka, Graduate Research Scholar (at IRRI from April 1980 to September 1980)
Mr. G.N. Nyamasyo, Graduate Research Scholar
Mr. J. Nyoike, Technical Assistant Trainee (at the Tata Institute of Fundamental Research, Bombay, India, from October 1979 to July 1980)
Mr. S.H. Okech, Senior Technician (at University of Philippines and IRRI since June 1979)
Miss Lucy Oketch, Graduate Research Scholar
Mr. J.C. Oleka, Senior Technician (at ICRISAT from September 1979 to March 1980)
Dr. J.O. Olobo, Graduate Research Scholar
Dr. T.O. Olova, Postdoctoral Research Fellow (at CSIRO, Australia since April 1980)
Mr. Patrick Oluya, Technical Assistant Trainee (at the Tata Institute of Fundamental Research, Bombay, India from August 1979 to May 1980)
Miss D. Sabwa, Graduate Research Scholar
Mr. J.C. Yarro, Graduate Research Scholar

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Miss S.M. Kagondu, Secretary
Mrs. Emma Kwach, Telephonist/Receptionist

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Mr. A. Okapesi, Assistant Head Cook
Mr. A. Lweya, Cook
Mr. P. Oponde, Kitchen Assistant
Mr. G. Gichuru, Kitchen Assistant

House-Keeping Staff

Miss J. Okodo, House-keeper
Mr. B. Randiga, Assistant Launder
Mr. P. Kibisu, Assistant Launder
Mr. C. Oyieyo, Room Steward
Mr. A. Mutwoli, Room Steward
Mr. L. Mulae, Room Steward
Mr. C. Okello, Gardener
Miss S. Machenje, Trainee

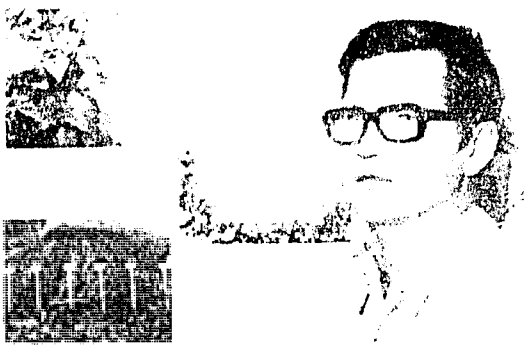
Professor William L. Nutting

Professor William L. Nutting, Programme Leader, Grassland Termites Research Programme, obtained his first degree at Harvard College in 1943. After a period in the U.S. navy he went to Harvard University where he earned his Ph.D. in 1950. From 1950 – 1955 he was a Research Fellow in Biology at Harvard and carried out research on the symbiosis between cockroaches, termites and their intestinal protozoans.

In 1955 he joined the Department of Entomology at the University of Arizona where he was involved in research and teaching. He carried out research on stored grain insects and on the biology, behaviour, ecology and control of termites in the Sonoran Desert and desert grasslands of South-Western U.S.A. and Mexico.

Professor Nutting's post at the ICIPE is his first experience in Africa and he considers it an opportunity of a lifetime for any biologist.

Dr. Z. T. Dabrowski

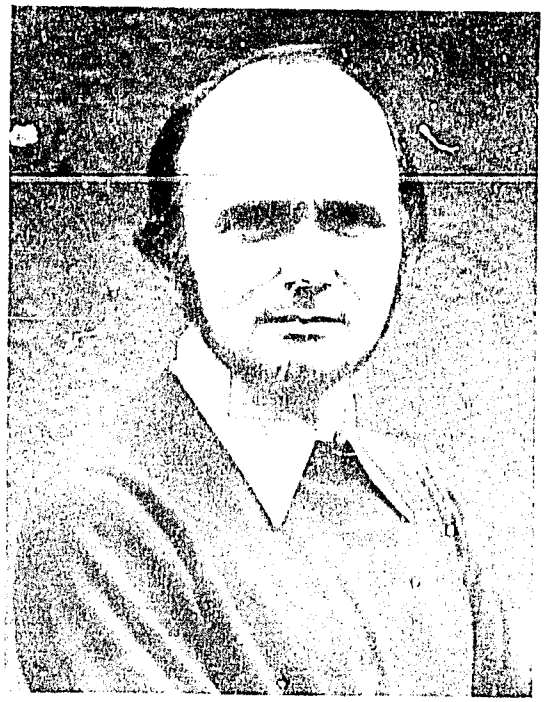


Dr. Z.T. Dabrowski, Programme Leader, Bases of Plant Resistance to Insect Attack Research Programme, was born in Poland and received his M.Sc. in 1964 and his Ph.D in 1968 at the Warsaw Agricultural University. His postdoctoral training was carried out in the Department of Entomology of the University of Kentucky, Lexington, U.S.A.

In 1973 Dr. Dabrowski went on a tour of laboratories in U.S.A. to study mass rearing of insects as related to the sterile male technique. He later received training at Moscow University and the Institute of Experimental Biology, Tallin, Estonia, U.S.S.R. This was followed by training at the Department of Animal Physiology, Agricultural University, Wageningen, Holland.

Dr. Dabrowski has been Associate Professor, Department of Applied Entomology, Warsaw Agricultural University and has led a Research Group working on Plant Resistance to Diseases and Pests, The Committee of Plant Protection, Polish Academy of Sciences. He is the Chairman, Section of Applied Entomology, The Polish Entomology Society.

Dr. Raymond Subra



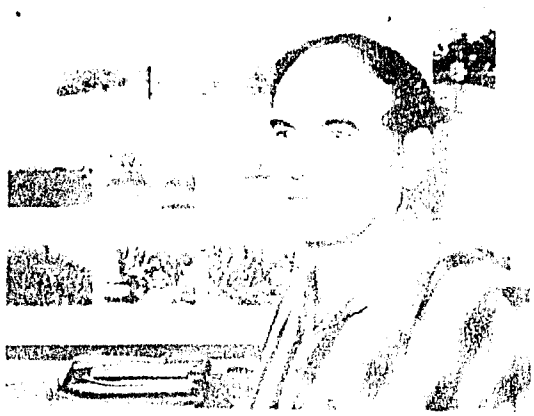
Dr. Raymond Subra, Programme Leader Medical Vectors Research Programme obtained his first degree from the Faculty of Science of the University of Toulouse, France in 1960. After two years as a trainee in ORSTOM and service in the French Army he was appointed as a Research Scientist with the ORSTOM Mission at Bobo Dioulasso in Upper Volta where he served for 6 years working on the ecology of mosquito vectors of Bancroftian filariasis.

Dr. Subra earned his D.Sc. from the South Paris University in 1971 and from that year to 1975 he was the Head of the Department of Medical Entomology at the ORSTOM Centre of Antananarivo in the Malagasy Republic. During this period he studied the ecology and distribution of malaria vectors and potential vectors of dengue. He also drew up a mosquito control programme for the Comoro Islands.

In 1975 Dr. Subra joined the ICIPE as a Research Scientist to work on the preimaginal ecology of the yellow fever mosquito along the Kenya Coast and was appointed Programme Leader in 1978.

Dr. Subra has been a WHO expert on vector biology and control since 1976.

Dr. Albert Challier



Dr. Albert Challier was born in France and educated at the University of Grenoble, where he studied zoology, botany and geology. He received training in medical entomology at ORSTOM for two years.

He later worked with the ORSTOM mission to OCCGE (an organization grouping 8 French speaking countries of West Africa with the aim of controlling various tropical diseases) in Bobo-Dioulasso in Upper Volta.

Dr. Challier was in West Africa for 17 years working on tsetse ecology and control and in 1974 was appointed as Head of ORSTOM Mission to OCCGE.

Dr. Challier's Ph.D. (Doctorat d'Etat-ès Sciences) was on "Ecology of *G. palpalis gambiensis* Van der Plank, 1949 (Diptera, Muscidae) in West African Savanna".

Dr. Challier joined the ICIPE in September 1978 as Programme Leader Tsetse Research Programme, and is a member of WHO Steering Committee on Epidemiology and Vector Biology and Control of African Trypanosomiasis as well as the FAO Expert Group on Programme for the Control of African Animal Trypanosomiasis and Related Development.

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PREFACE

SCIENTIFIC DEVELOPMENT: PLANNING FOR THE FUTURE

Twelve years ago, when the planning conference for considering the establishment of an International Centre of Insect Physiology and Ecology (ICIPE) met in October 1969 in Nairobi, it made specific recommendations that the institute be established in Nairobi as soon as possible, and that "the ICIPE be a centre of advanced research meeting the highest standards of world science; that the Centre should have close co-operation with the scientific community in Africa and thus contribute to the development of the latter; that the Centre should have as one of its central aims the making of discoveries and inventions that might lead to the design of new methods for insect pest control; and that the research project carried out at the ICIPE should capitalise on the wealth of research opportunities that exist in the insect and plant life of eastern Africa" (Preface to The ICIPE: Statement of Its Objectives, Activities, and Governance, March 1980). In the eight years since the ICIPE became functional from very small beginnings in a small converted car-port, the ICIPE has gradually brought into realisation this four-pronged priority package of its work programme, institutional building, and scientific linkages.

The many international evaluation missions the ICIPE has received since 1974 — culminating in a veritable flood in the last two years, 7 in 1979 and 9 in 1980 — have all testified at some length on the quality of ICIPE's research, of its vigorous intellectual environment, and of its concentration on development-oriented research of high relevance to pan-tropical regions of the world. The careful selection of the ICIPE staff on a world-wide basis, linked to the scientific cooperation the ICIPE has always maintained with the leading academies of science with interest and expertise in insect science, and ICIPE's insistence on having a flow of senior visiting scientists to the Centre (through its periodic appointment of Research Advisors, Research Consultants, Scientists-in-Residence and Research Associates in critical areas of scientific enquiry) has ensured that the ICIPE maintains a new-land frontier outlook in each of its programme activities. This frontier-manship, as well as ICIPE's highly intensified multi-disciplinary approach to pest management research — in ecology, ethology, bionomics, epidemiology, agronomy, veterinary science, parasitology, natural products chemistry, cell-biology, endocrinology, insect pathology, sensory physiology, and many other disciplines all targeted on specific insect problem-areas — has given the ICIPE a high profile in Africa, and has — engandered a great deal of collaborative activities. ICIPE's training programmes reflect the considerable degree of utilization of ICIPE's training facilities by African institutions and governments. Several Research Associates from several African countries (Nigeria, Ghana, and Kenya) are working in the ICIPE from time to time over a period of 3 to 4 years in each case. Some African research institutions have cooperative programmes with the ICIPE, for example the West African Rice Development Association (on rice pests) and the Uganda Trypanosomiasis Research Organization (on tsetse and trypanosomiasis). And, the ICIPE has helped to find in December 1978 the African Association of Insect Scientists, and the two together have sponsored the establishment of a new international tropical science journal INSECT SCIENCE AND ITS APPLICATION, which Pergamon Press began publishing on a quarterly basis in October 1980. The title of this journal, as well as ICIPE's own core programme — which is based on six groups of some of the most difficult tropical pest problems at this time (crop borers, armyworm, grassland termites, livestock ticks, tsetse, and medical vectors), as well as a prime area essential for basic plant protection for the peasant farmer, namely bases of plant resistance to insect attack — attest to ICIPE's commitment to pest management research, while insisting on the highest scientific standard of enquiry and interpretation.

The first few years of its existence has seen the concentration of the ICIPE on Africa. However, from 1978, the ICIPE has gradually seen its role as also encompassing a wider regional approach — that of a pan-tropical outlook on a selected group of scientific endeavours. For instance, the ICIPE has a small team of researchers located in the Philippines, working closely with the International Rice Research Institute on the rice brown planthopper, especially on questions related to plant resistance to this important pest and development of planthopper biotypes that can colonise erstwhile resistant cultivars. The Centre's training programme, particularly at the graduate and practitioner levels have always been pan-tropical. We expect these, and other pan-tropical linkages, to become more extensive in the years to come.

We believe that our preeminent goal in the first decade of ICIPE's existence has now been accomplished — that of establishing a critical mass of talented, highly motivated, interacting scientific community at the ICIPE all cooperatively targeted on a few, carefully selected pest management goals, collaborating productively with the wider scientific and practitioner community in Africa and other tropical regions, and concentrating on the major pest problems of its constituency. The next phase for us now seems to be one of giving this ICIPE scientific community the means to accomplish its mandate on a continuing and rationalised basis.

The means include modest but well-designed laboratories, relevant equipment and adequate supporting services, basic field research facilities to meet the varied ecological and agronomic requirements, and modest but stable financial resources to satisfy the alimentary needs of the institute. The emphasis is on modesty — in terms of facilities and the size of the institute. The scientific standards of the ICIPE will, however, always be on an ascendant scale. We very much hope that our original sponsors, together with our new donors, will continue to support us in this difficult but most rewarding task in the service of tropical developing world.

THOMAS R. ODHIAMBO
Director, ICIPE

14th February 1981

teaching or in research institutions to undertake advanced research in the special research environment found at the ICIPE.

(v) To improve the communication of its staff by organizing language courses.

Over the last 10 years, several training programmes have been evolved and continue to be reviewed and rationalized. In 1980, for the first time, training received an in-depth review at the ICIPE Annual Research Conference, held in June. In addition, several review missions from the ICIPE Donor Agencies undertook extensive reviews of the division, alongside the research activities. Arising from the recommendations of the various reviews, the Centre is set for further rationalisation of its training programmes and policies.

Achievements in 1980

Achievements in training for the different programmes can be gleaned from Table 1.

Table 1. Training Output (man-years) at ICIPE Research Centre, 1980

Type of Training	No. of man-years
High School Bursary	5.00
Postgraduate Training	14.00
Research Training Internship	6.00
Visiting Research Associates	2.00
Postdoctoral Training	15.00
Specialized Training	7.00
Technical Training	4.00
Management Training	4.00
Secretarial Training	1.58
Short Courses	1.50
Language Courses	4.00
Total	64.08

High-level research training comprised about 60% of all the training at the Centre in 1980; these were in the areas of postgraduate, postdoctoral and other research internships. Participants to these programmes came mainly from Nigeria, Ghana, Uganda, Tanzania, Zambia, Malawi, Sudan, Sri Lanka, Somali and Senegal.

The short course for practitioners in Components Essential for Ecologically Sound Pest and Vector Management Systems, co-sponsored by the ICIPE and the United Nations Environment Programme, entered its fourth year. Twenty six (26) participants from Brazil, Ethiopia, Israel, Kenya, Malawi, Mauritius, Nigeria, Philippines, Swaziland, Tanzania, Uganda, and Zambia attended this 3-week course, which was held from 30th June to 20th July 1980. The other short course conducted at the ICIPE from 24th March to 4th April 1980, for training of internal evaluators from institutions benefiting from long-term grant of the UNDP World Bank/WHO Special Programme for Research and Training in Tropical Diseases. This training, which was attended by nine participants from Argentina, Brazil, Cuba, Indonesia, Kenya, Malaysia, Mozambique and Philippines, used the ICIPE internal evaluation of its Medical Vectors Research Programme, a recipient of the Special Programme grant, as a case study.

The Centre also held four important International Study Workshops as follows:

1. The Use of Naturally Occurring Plant Products in Pest Control, Nairobi, from 12th to 15th May 1980.
2. Sorghum Shootfly, Nairobi, from 5th to 8th May 1980.
3. Cereal Stem-borers and Legume Pod-borers, Mbita Point Field Station and Nairobi, from 1st to 6th September 1980.
4. The Promotion of Training of Insect Scientists in Africa, Lusaka, Zambia, from 1st to 5th December 1980.

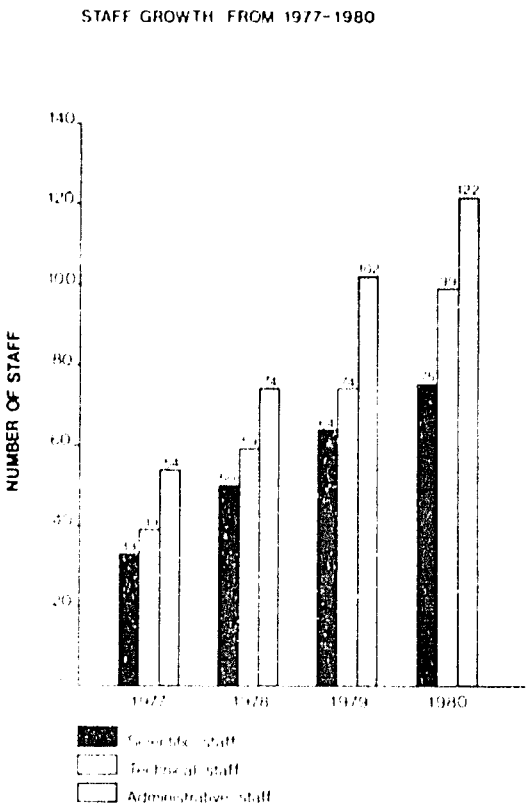
A total of 98 scientists from 19 countries, mainly of the developing tropical world, attended these workshops.

Seminar deliveries continued at both the headquarters in Nairobi and the field stations; a total of 37 of these were conducted.

ADMINISTRATION

Staff Growth

ICIPE Staff has grown from 126 in January 1977 to 296 in December 1980. This represents an increase of 135% which gives an average growth rate of 45% per annum. The staff categories have been divided into the Scientific, Technical, and Administrative Staff as shown in the attached histogram.



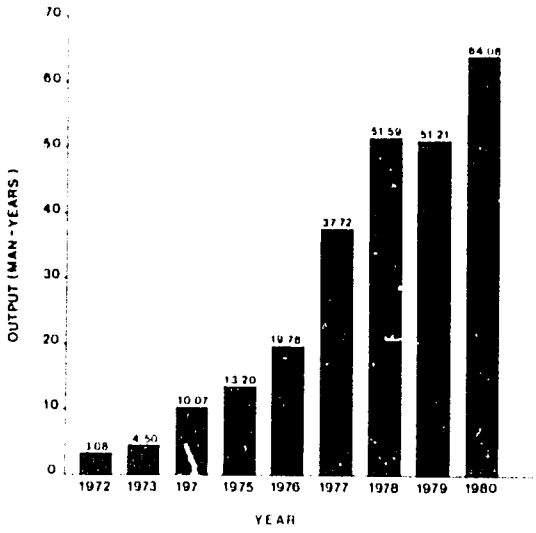
Scientific staff includes Postdoctoral Research Fellows, Scientific Research Officers, Associate Scientific Officers, Research Assistants and Graduate Research Scholars.

Administrative staff includes all staff in the Office of the Director, Communication and Training Division, Finance Division, Personnel and Office Management Division, Duduville International Guest Centre and Field Stations.

Technical staff refers to those members of staff who render technical support to Scientific staff and includes Principal Technicians, Senior Technicians, Technicians, Junior Technicians, Technical Assistants and Technical Assistant Drivers. Staff in the Workshops and Transport Unit loosely fall under this category.

TRAINING AT THE ICIPE

In 1980, the Centre marked its 10th Anniversary, and fittingly registered a quantum jump in its training activity. There was 50% increase in the Centre's output, from 42 man-years in 1979 to 64 man-years in 1980 as shown in Fig. 1.



*Total output for the 9 years is 261.23 man-years

Fig. 1. Training output (in man-years) at ICIPE Research Centre, 1972 – 1980.

In 1972 when the ICIPE became a functional entity, only 3 man-years of training was achieved; and the current output represents a twenty-fold increase.

The ICIPE envisages an even larger increase over the next five years; therefore in looking toward the next decade, it would be fitting to recapitulate on the objectives of training at the ICIPE. Research and training comprise the dual mission of the ICIPE; but training has an overall objective to build scientific and technological capabilities of developing countries. Within that broad objective, the specific ones are summarised as:

(i) To provide advanced training in research methodology and application for promising graduate and postdoctoral research fellows.

(ii) To acquaint young scientists, actively engaged in pest and vector management and/or who are starting their careers with recent advances in components essential for ecologically sound methods to pest and vector control.

(iii) To provide an international forum for the discussion and exchange of knowledge among scientists through seminars, study workshops, symposia, and conferences.

(iv) To allow young scientists who are either

PROGRAMME ON BASES OF PLANT RESISTANCE TO INSECT ATTACK

Programme Leader

Dr. Z.T. Dabrowski (1979)

Research Staff

Dr. R.C. Saxena (1977) Senior Research Scientist
Dr. R.S. Ochieng (1977) Research Scientist

Mr. E.O. Omolo (1978) Agronomist
Mr. E.O. Arap (1980) Technician
Mr. E.O. Nyangiri (1979) Senior Technician
Mr. S.O. Obiero (1980) Technician
Mr. S.H. Oketch (1977) Technician, IRRI, Philippines
Mr. F.O. Onyango (1979) Technician
Mr. M.D. Bungu (1980) Junior Technician
Mr. E.L. Kidhavai (1980) Junior Technician
Mr. P.E. Njoroge (1980) Junior Technician
Mr. M.O. Arwa (1980) Technical Assistant
Mrs. A. Ragot (1978) Technical Assistant
Mr. P. Otieno (1980) Subordinate Assistant

Collaborators

Prof. E. Smith, Crop Borers Research Programme
Dr. A.K. Raina, Sorghum Shootfly Research Project
Dr. K.V. Seshu Reddy, Crop Borers Research Programme
Dr. W.A. Otieno, Insect Pathology
Mrs. D. Sabwa, Insect Pathology
Mr. N.Y. Patel, Salivary Gland Physiology
Dr. D. Whitehead, Chemistry and Biochemistry
Dr. I. Gebreyesus, Biometry
Mr. A.J. Leaney, Insect and Animal Breeding
Dr. J. Clark, Sensory Physiology
Dr. S.R. Singh, IITA, Ibadan, Nigeria
Dr. L.G.N. Jackai, IITA, Ibadan, Nigeria

Introduction

Z.T. Dabrowski

In 1980 research on plant resistance concentrated on five aspects: (1) confirmation and expression of insect resistance in ICRISAT sorghum lines, IITA cowpea lines, IRRI rice lines; in maize lines originated from CIMMYT and the Kenya National Programme (E.O. Omolo and Z.T. Dabrowski); (2) studies on mechanisms of plant resistance to insects including behavioural and physiological relationships between pest and crop plant tested (Z.T. Dabrowski); (3) Physiological and behavioural mechanisms responsible for development of new insect biotypes on resistant lines (R.C. Saxena); (4) experimental bases of insect mass rearing for screening purposes (R.S. Ochieng); and (5) agronomic aspects of pest status on moderate resistant cultivars under intercropping systems on small farmers' fields (E.O. Omolo).

Close co-operation and joint research projects with collaborating institutions (The International Institute of Tropical Agriculture, Ibadan, Nigeria; The International Rice Research Institute, Manila, Philippines; The International Crop Research Institute for the Semi-Arid Tropics, Hyderabad, India; The International Maize and Wheat Improvement Centre, Londres, Mexico; Texas Agricultural Experimental Station, Lubbock, U.S.A.; Kenyan National Agricultural Research Station and FAO Sorghum Regional Programme in Kenya) eliminated the necessity of preliminary screening on a large scale of hundreds of cultivars for insect resistance at the Mbita Point Field Station.

Resistance of Maize to Stem Borers

Z.T. Dabrowski, E.O. Omolo and E.O. Nyangiri

Our observations on the spatial distribution of *Chilo partellus* and *Eldana saccharina* egg masses on maize plants in different development stages showed that most of the eggs were laid on the 2nd and 3rd leaf of maize plants of stage 4 and 5, and on the 5th leaf of stage 6 and 7 (Fig. 1). A significantly less number of eggs were laid on plants in later stages. This phenomenon was explored in a following experiment conducted on the oviposition behaviour of *Chilo* females on three maize lines under screen-house conditions.

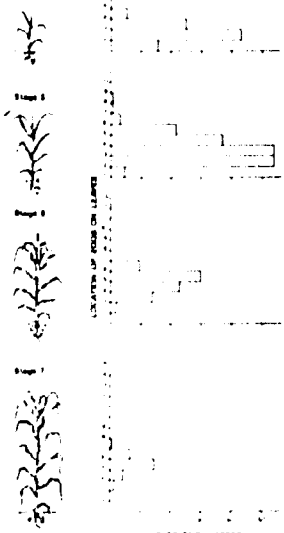


Fig. 1: Distribution of *Chilo partellus* and *Eldana saccharina* egg masses on leaves of maize at different stages of growth. Mbita Point Experimental Farm, December 1979 – February 1980.

Inbred lines used for breeding of Kitale Synthetic II maize cultivar grown in Kenya, showed differences in their response to stem borers infestation. Inbred D and G were identified as resistant and inbred A as highly sensitive. Preliminary observations on females' oviposition behaviour suggested that non-acceptance for oviposition was an important type of resistance against stem borers in maize. The preference for oviposition was measured by the number of eggs laid by *Chilo partellus* females. Artificial infestation of the plants in their 5th developmental stage was done by releasing 35 pairs of *Chilo* moths in each of the four screenhouses in the Mbita Point Field Station.

The number of eggs laid on each plant was counted separately and the leaf damage level assessed at weekly intervals for one month starting from one week after infestation of eggs. Thirty days after infestation evaluation of the overall plant damage was carried out. Leaf damage and plant damage evaluation was based on the injury level 1 – 9 rating system.

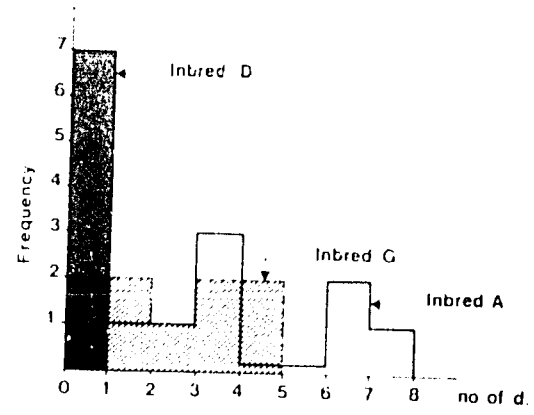


Fig. 2: Distribution of number of completely destroyed plants per row of 14 plants. Mbita Point Field Station, July 1980

Table 1: Infestation of Three Maize Lines by *Chilo partellus* Under Screenhouse Conditions in Non-choice Situation

LINE	NO. OF EGGS PER PLANT	LEAF DAMAGE 1 WEEK	2 WEEK	3 WEEK	4 WEEK	OVERALL PLANT DAMAGE (after 4 weeks)	NO. OF TUNNELS	NO. OF LARVAE	NO. OF PUPA
Inbred A	13.3	1.7	2.8	8.9	9.0	6.3	5.0	11.0	4.0
Inbred D	11.0	1.4	2.7	3.3	3.6	3.8	3.3	6.4	2.7
Inbred G	10.0	1.6	3.1	1.0	5.1	5.5	2.7	8.9	0.2
In the choice situation									
Inbred A	17.9	2.4	7.5	8.9	9.0	3.0	Not sufficient number of plants in the row		
Inbred D	9.1	1.2	1.6	2.8	5.2	7.5	–	–	–
Inbred G	7.8	1.1	1.6	1.9	5.8	5.2	–	–	–

A significant difference in the number of *Chilo* eggs laid on the susceptible (Inbred A) and the other two lines tested was found in the choice and non-choice situations. On the average 13.3 eggs per plant were found on the susceptible line A and only 10 – 11.0 on Inbred G and D, respectively. In the greenhouse, where the three lines tested were grown on adjacent rows, a much greater number of eggs was found on Inbred A than on the moderate resistant lines (Table 1). Overall plant damage was also higher for all cultivars tested in the choice test.

Preliminary observations on females' behaviour suggest that the maize lines which are hairy on the upper part of the leaf (Inbred D, G) were less oviposited, but were as much oviposited as the others on the lower surface of the leaf, which is glabrous in all lines. Pilosity or some unknown factor associated with it, has a negative influence on oviposition. The number of larvae developed in susceptible line A was 30% higher than in the resistant lines, but the destructive effect of larval feeding in stems on plant development of Inbred A was considerably higher than on Inbred D and

G. All plants of Inbred D survived the stem borers attack, whereas 6–8 out of a total of 14 plants of Inbred A grown in rows, were destroyed by larvae. Distribution of the number of plants destroyed by stem borers in the subsequent rows of three lines is presented in Fig. 2. It was found that the restriction of damage indexes only to number of larvae in the stem; number and length of tunnels in stems at harvest and the average yield/plant did not give a full picture of stem borers-maize relationships when resistant, tolerant and highly susceptible maize lines are screened. Some other indexes, as the proportional frequency distribution of infested plants in replicated plots or rows should also be calculated. The high susceptibility of Inbred A is therefore related to higher oviposition on the upper part of the leaf and to higher sensibility of plants to larval feeding in stem.

An experiment planted in October is related to studies on larval behaviour on leaves and in stems of lines tested and to the effect of feeding of consecutive larval instars in stems of different developmental stages on plant's survival and on the formation of cobs.

A new bioassay technique is also proposed to measure the effect of allelochemicals negatively affecting larval feeding (feeding deterrents or inhibitors) in resistant cultivars. Glass tubes 4mm in diameter and 10 cm in length are filled with 4% agar-cellulose gel and the individual larvae are released at one end of the tubes. The extent of feeding is measured after 24 or 48 hours and expressed in mm of tunnelling in the tube.

One wonders why maize breeders in research stations are using the sensitive line for commercial hybrid production. The national breeding programmes in their package recommendation included the application of insecticides to protect plants against pest attack and the differences in maize infestation by stem borers are not identified under such chemical "umbrella."

Inbred A is a high yielding line, possessing big cobs reflected by the number of rows per cob and has a very good seed quality. Our studies on maize resistance to insect attack prompted the interest of the Kenyan breeders to the fact that some of their lines showed moderate resistance to stem borers.

Screening Maize Lines for Resistance to Stem Borers

E.O. Omolo

The programme to screen maize for stem-borer resistance is made up of two parts: the first part being that of evaluating both local and introduced cultivars for their resistance to stem-borers. Those that show acceptable resistance would be used in a breeding programme to form or widen the genetic base of the main breeding population. The second part is a comprehensive breeding system aimed at selection and development of the maize genotypes resistant to stem borers from the two basic breeding populations of maize in Kenya. When sources of new or higher levels of resistance are identified, the lines will be released to national programmes in East Africa to be included in breeding of new locally adaptive and insect resistant commercial cultivars.

S_1 progeny testing method, where selected S_1 cobs from a bulk planting of populations, is the starting point for selection of S_1 progeny. Variation among S_1 progeny is normally greater than among ear to row or test cross rows. There is also a minimum of 50% of loci which are homozygous, there is, therefore, no masking effect of the tester. Since the time given is limited the method fits our purpose because it requires three seasons to complete one cycle of the recurrent selection.

One hundred S_1 families selected from an early maturing variety Katumani, grown mainly in the marginal dry lands of Kenya, were progeny tested in a triple 10 x 10 lattice replicated trial during the long rain season of 1980. Based on the leaf/plant damage level; number of larvae and pupae per plant — the tunnelling per plant and yield per plant, 19 tolerant and 6 sensitive lines were identified for further testing and confirmation in a replicated trial during the main season in 1981 (Tab. 2). Screening was done under moderate natural infestation. Significant correlation between the number of borers and tunnelling on one side and a similar significant correlation between resistance and grain yield was observed. Since our selection is farmer oriented, only high yielding tolerant lines were selected for further studies. Only for experimental purposes, low yielding but high resistant lines (Line: 29, 27; Tab. 2) were chosen to form Katumbita lines. Two thousand individual plants of Kitale synthetic II were planted in April 1980. Kitale is a late maturing variety from which most of the parental lines of Kenya hybrids were drawn. Since Inbred A, G and D were developed by Kitale breeders from this variety, it was felt that a further follow-up would yield more information on the population or indicate other lines that might not have been identified at the first attempt.

The purpose of screening Kitale II population for resistance, is to cross the Katumani and Kitale II resistant lines to come up with a medium maturing, sub humid tropical type for release in the Lake Victoria region. Twenty five lines showing low level of infestation by borers and five sensitive lines have been identified as the Kitambi lines. Lines with minimal number of borers and short length of tunnelling were considered much more resistant than those with higher number of larvae and longer tunnelling. Tunnelling was a better estimation for resistance than the number of larvae collected, because at harvest only larvae of the last generation of stem-borers may be found in stems.

Table 2. Infestation of Selected Katumani Lines by Stem Borers, Mbita Point Field Station, 1980

Line	% Infestation	Stem Tunnelling by larvae	No. of larvae per plant	Cob length (cm)	No. of row/cob	Grain yield per plant (g)
TOLERANT						
22	8.8	10.0	0	12.3	11.6	417.1
63	1.4	19.0	1	14.0	11.7	880.4
23	9.3	25.0	1	14.9	12.3	1186.5
43	7.5	27.0	3	11.8	9.7	729.0
44	3.0	58.0	0	13.6	11.6	739.3
27	0.0	62.0	1	15.0	11.1	568.9
20	8.8	65.0	0	13.0	13.0	1094.8
16	3.9	69.0	0	14.3	14.8	848.8
6	5.6	74.0	5	12.7	12.0	791.0
47	6.1	75.0	2	12.4	13.6	891.7
19	0.0	76.0	1	12.4	11.8	1080.8
25	8.6	89.0	11	13.4	11.6	860.1
76	9.2	87.0	0	13.3	11.8	940.0
33	9.4	213.0	6	13.0	11.1	921.8
12	8.1	261.0	4	13.0	12.0	1112.5
10	5.6	334.0	7	8.6	12.0	878.9
18	3.0	350.0	12	13.8	12.3	829.0
28	0.0	409.0	14	13.6	12.0	1080.9
35	5.4	433.0	22	12.9	10.6	728.2
SENSITIVE						
53	5.4	170.0	4	7.5	10.5	367.7
30	10.0	278.0	12	8.7	10.0	179.5
35	6.3	331.0	2	10.2	10.4	350.8
7	5.7	347.0	31	11.3	11.8	77.6
4	5.6	450.0	38	9.3	10.9	176.9
84	5.6	527.0	62	12.2	10.8	181.0

A total of 460 families of maize from the CIMMYT advanced populations were planted in the short rain season in September 1980. The evaluation of their response to stem-borers (*Chilo*, *Eldana* and *Busseola*) is based on leaf feeding ratings (1–9 scale), overall plant damage ratings (1–9 scale), numbers of tunnels; numbers of larvae and pupae in stems. A number of them do show some promise, however, potassium deficiency in our soils has caused a lot of streak yellows on many plants that could easily be mistaken for virus streak.

Sorghum Resistance to Stem Borers

Z.T. Dabrowski, E.L. Kidavar and M.O. Atwa

Four different collections of ICRISAT lines previously identified as resistant to *Chilo* infestation under Indian field conditions were planted in March and later resown in May in a randomised complete block design replicated six times to eliminate the effect of lack of uniformity in insect distribution upon the expression of resistance under field conditions. The following observations were made:

- counting of *C. partellus* egg masses in three day intervals and noting the position of eggs on leaves and stems;
- counting of "dead hearts" caused by *Chilo* larvae on young seedlings;
- evaluation of leaf damage caused by first instar *Chilo* larvae at weekly intervals during a month of infestation by first egg masses;
- evaluation of overall plant damage level according to 1–9 rating scale at two weeks intervals;
- counting of number of tunnels, total number of cm of tunnelling (and plant height), number of larvae and pupae of *Chilo* and *Eldana saccharina* present in the split plants during harvesting.

Significant differences in the percentage of plants infested with stem borer eggs were found between ICRISAT lines. Lines E302, E303, IS2209, IS1044, IS1151 showed the lowest infestation, in comparison to 50% for Serena or 53.8% for IS12611 (Tab. 3). The number of plants showing infestation by larvae was independent of the location of replicates for most cultivars, which suggests uniform infestation in the experimental field. Comparison of the infestation of different lines by stem borer eggs with the plant damage suggests that non-acceptance is one of the mechanisms of resistance. Only two line – IS2209

and IS10262 showed significantly lower numbers of holes/stem and numbers of larvae feeding in stems (Table 3). The IS2205 line showed the highest number of larvae/stem (5.0) and the highest average overall plant damage. In most of the lines tested, it was observed that there was a close correlation between overall plant damage, number of holes and larvae except in the case of IS1044. The number of larvae and holes in stems was high, but leaf and plant damage was significantly lower than most of other cultivars included in the experiment. It is suggested that larval feeding on leaves was limited or shorter and they tend to move earlier to stems on the lines showing low leaf damage.

Point conditions. The short height and the uniformity of the lines tested have attracted a lot of interest from local farmers around Mbita. The percentage of plants showing infestation by stem borer larvae has varied from 10 to 45.0%, but the average of plant damaged, reflected by the extent of larval feeding on leaves, was low.

At harvest, nearly all of the lines tested showed high stalk damage by larvae and extensive larval feeding on leaf sheaths closely attached to the stalk and therefore unnoticeable during the ratings of leaf and plant damage. The yield of most of the lines was high, suggesting a tolerance mechanism to *Chilo* and *Eldana* attack in these lines. The FAO Sorghum Improvement Programme

Table 3. The ICRISAT International Stem Borers Nurseries, Mbita Point Field Station, 1980.

Cultivar	Stem Borers							Shootfly
	% Plants with eggs	% Plants damaged	Leaf damage	Overall plant damage	No. of hole/ stem	No. of larvae/ stem	No. of pupae/ stem	% "Dead hearts"
E 302	24.8	33.6	1.5	2.3	16.1	3.5	0.5	32.4
IS 2209	21.5	35.3	1.5	2.3	6.1	1.7	0.5	64.6
IS 1041	16.7	40.2	1.5	1.8	19.9	3.5	0.6	22.5
E 303	20.2	41.4	1.5	2.3	13.4	3.3	0.9	22.8
IS 10262	28.6	43.9	1.5	2.3	7.3	2.0	0.6	58.9
IS 8844	36.7	52.8	1.8	3.0	14.6	3.6	0.7	58.3
IS 3788	29.4	53.0	2.3	3.2	13.2	3.9	1.2	29.9
CSH–1	33.3	56.7	1.8	2.8	14.3	3.8	0.7	58.9
IS 1151	12.8	58.6	2.2	2.8	12.7	2.9	0.9	32.5
CS 3591	28.3	59.2	1.8	3.0	11.2	3.9	1.1	31.7
IS 12447	28.9	61.8	1.5	2.7	11.0	2.4	0.4	60.1
CHR 148	28.0	64.9	1.8	3.8	11.7	3.8	0.4	39.0
SERENA	50.4	65.8	2.2	3.8	17.4	4.4	1.2	39.7
IS 1357	37.6	67.4	2.0	3.2	17.6	3.9	0.8	60.7
IS 8884	31.4	70.2	1.8	3.0	14.6	3.6	0.7	58.3
IS 1082	33.3	72.0	2.3	4.3	20.0	3.4	0.8	8.3
IS 2205	40.0	82.5	2.0	9.5	17.1	5.0	0.9	20.8
IS 12611	53.8	87.8	2.2	5.5	11.9	2.7	0.8	41.0
IS 2122	30.0	89.1	2.0	4.2	13.1	3.8	0.8	22.5

Forty sorghum lines identified as resistant to aphids and to the sorghum midge, *Contarinia sorghicola* in the USDA Sorghum Improvement Programme, were also included in our screening. Observations made in India, North and South America, and Africa gave clear indications that the damage due to the midge increased significantly during the last years. Development of multiple resistance in sorghum in tropical countries to various pests requires the identification of sources of resistance not only to the present major pest species, but has to take under consideration the species whose importance as sorghum pests is increasing to prevent future outbreak on new introduced cultivars.

In cooperation with Dr. Johnson (TARS, Lubbock, Texas, USA), an attempt was made to screen lines that had already been identified as resistant to other pest species and are suitable to arid areas. The lines, originated from Ethiopia, showed high tolerance to drought under Mbita

in Kenya has already included some of the lines selected by us as tolerant to stem borers and drought under Western Kenya conditions in their breeding project of new sorghum cultivars suitable for semi-arid tropics.

On the basis of our experiments conducted in 1980, some changes in the evaluation of sorghum response to stem borers' attack were introduced. Analyses of all sorghum plants after harvesting showed that there was close correlation between the number of tunnels and the number of exit holes caused by *Chilo* and *Eldana* larvae in the stems. While dissecting the stalk and measuring the length of tunnelling is probably the most accurate method of assessing damage by stem borers' larvae, it requires six times the time required for counting holes. Perhaps when future selections are to be made from among genotypes with relatively high resistance, tunnels will need to be measured. In initial evaluations, however, only few genotypes have been found to be resi-

stant. Counting holes rather than measuring tunnel length greatly reduced the time required for making initial evaluations.

All experiments on sorghum resistance to *Chilo partellus* and *Eldana saccharina* suggest that there are five levels of relationships affecting plant colonization and damage level:

- non-acceptance for oviposition
- movement of first larval instars on plant
- feeding of young larvae on leaves or leaf sheaths
- feeding of larvae on young plants causing "dead hearts"
- tunnelling of older insect instars in stems
- plant tolerance to leaf and/or stem infestation.

Results of field observations on larval behaviour on different sorghum lines planted in September, previously described by us as resistant, tolerant and susceptible, are still being collected. The initial number of eggs and their position on the plant is noted, and the number of larvae feeding on leaves, on leaf sheaths adhering to stems and in the stems are later counted at seven day intervals to rate the survival of larvae on different levels of sorghum — *Chilo* and *Eldana* relationships. Preliminary observations showed that on some tolerant lines (but not resistant ones), *Chilo* and *Eldana* larvae concentrate their feeding on the leaf sheaths adhering to stems, and not on leaf blades or in stems.

New experiments are planned to separate the two different phenomena involved in larval feeding on tall sorghum lines with leaves widely detached from the stalk (average lower infestation) and on some short lines with wide leaf sheaths composing cohesive layers around the stem (high stem infestation). The differences in stalk tunnelling and in number of larvae feeding on leaf sheaths may be caused by larvae escaping predation by formicids on the short lines, and not by biophysical and or biochemical properties of lines tested. Field observations by Greathead (1970) and Girling (1978) showed 90–93% destruction of stem borer eggs and of first larval instars by formicids. Short type sorghum plants can secure preferred "ecological niche" ensuring higher survival of stem borer larvae and therefore are more liable to damage.

Preliminary Experiments on Cowpea Resistance to *M. testulalis*

Z.T. Dabrowski, M. Bungu, E.O. Arigi and P. Njoroge

As a result of previous work by Dr. S.R. Singh and Dr. L.G.N. Jackai which showed the existence of cowpea resistance to the legume pod borer, three cowpea lines of IITA origin (Vita I, Ife Brown and TVu 946) were selected for the study on mechanisms of resistance to *Maruca*.

These were planted in a randomized block design in plots measuring 5x3m, replicated four times in four screenhouses size 5 x 10 x 2.5m. Twenty five days after planting, plants in the pre-flowering stage were artificially infested with first instar larvae (5 larvae per plant). The number of terminal shoots damaged and number of larvae found on the plants, were counted at three day intervals. The TVu 946 plants showed the lowest infestations, and Vita I plants the highest ones (Tab. 4). Four times more larvae were found on Vita I than on TVu 946 plants on the 10th day after infestation.

In addition TVu 946 also had significantly fewer damaged flowers and pods. The plants of this cultivar have important characters that make them less susceptible to flower and pod damage: they have abnormally long peduncles; the pods, about two per peduncle, are held well over the plant canopy at a wider angle than normal varieties and, therefore, escape considerable flower and pod damage. TVu 946 is closer to the "wild type" cowpea, but it has early maturity and may be an important parent in a cowpea improvement programme. Studies on mechanisms of TVu 946 resistance to *Maruca* have been initiated with observations on food selection and acceptance of the third and fourth instars of *Maruca* larvae. Terminal shoots, flowers and pods of TVu 946 cowpea lines possess some unidentified characteristics negatively affecting *Maruca* feeding behaviour (Tab. 5).

Plants in different growing stages should be tested separately because differences in the growing pattern of cultivars tested affect oviposition and

Table 4. Preference of *Maruca testulalis* larvae towards terminal shoots flowers and pods of three IITA cowpea lines (total numbers of larvae after 15, 30, 41, 60 and 75 min)

Numbers of Insects	TERMINAL SHOOTS			FLOWERS			PODS		
	Vita 1	Ife Brown	TVu 946	Vita 1	Ife Brown	TVu 946	Vita 1	Ife Brown	TVu 946
Third larval instar	48	40	28	47	33	22	59	53	19
Fourth larval instar	58	41	33	37	19	15	39	35	18

Table 5. Number of *Maruca testulalis* larvae, and stems damaged of three IITA cowpea cultivars after artificial infestation with 5 larvae/plant on September 3, 1980

Date	Number of stems damaged			Number of larvae		
	VITA 1	IFE BROWN	TVu 946	VITA 1	IFE BROWN	TVu 946
Sept. 5	21	34	22	40	53	24
Sept. 8	63	11	5	49	18	1
Sept. 11	34	38	4	15	10	0
Sept. 14	18	24	2	5	2	0
TOTAL	136	107	33	109	83	25

larval development. These tests should help to distinguish between host evasion and indigenous resistance.

The present work on the mechanisms of cowpea resistance to *Maruca* is concentrated on the oviposition behaviour of moths on different cowpea lines and on the role of biophysical and chemical factors present in the resistant lines influencing *Maruca* behaviour and physiology.

Investigations on Physiological Components of *Atherigona soccata* Larvae and Sorghum Interaction

Z.T. Dabrowski and N.Y. Patel

Resistance in sorghum to the shootfly is well documented, but studies on the mechanisms of resistance are limited only to a few publications. Our investigations on the nature of resistance found in some sorghum cultivars selected by ICRISAT, India, are concentrated on identification of plant properties involved in antibiosis. Comparison of number of eggs laid by shootflies on different sorghum lines with the percentage of plants showing typical "dead hearts" with larvae feeding inside clearly indicate, that the mortality of newly hatched larvae is high on some cultivars (Tab. 6). The assumption was made that for a complete understanding of the biochemical and physiological effects of various sorghum cultivars upon the development of *A. soccata* larvae, it is necessary to investigate the relationships between plant chemicals occurring in the growing and decaying sorghum tissues and the digestive ensymes of larvae. Changes in food quality in the infested seedling observed during the development of larva should be reflected in changes in the activity of insect enzymes. Knowledge of the digestive enzyme activity of the shootfly larvae could explain the mechanisms of insect adaptation to the particular type of food affecting the nutrition of larvae. In this respect an introduction to the anatomical organization of the alimentary canal of larvae is needed to understand the digestion and absorption of food taken from resistant and susceptible sorghum lines. The present preliminary investigation was undertaken to determine qualitatively some of the enzymes present in the gut and salivary glands of third instar larvae by locating enzymes histochemically on polycrylamide gels.

So far, there is no information available on the presence of various enzymes in the gut and salivary glands and on the nutritional requirements of *A. soccata* larvae. The alimentary canal of the third

Table 6. The ICRISAT International Sorghum Shootfly Nurseries, Mbita Point Field Station, 1980

Cultivar	% of plant with eggs		% of "dead hearts"	
	Main shoots	Tillers	Main shoots	Tillers
IS 4660	31.8	100.0	4.5	40.0
IS 2291	22.0	25.0	7.3	25.0
IS 2263	26.3	36.4	12.5	18.2
IS 17739	34.1	23.1	12.2	38.5
IS 18321	85.4	52.1	14.6	16.6
IS 18363	41.5	69.3	14.6	15.3
IS 18427	47.6	62.1	16.7	44.4
IS 2162	26.2	54.4	17.0	36.3
IS 18361	26.8	37.5	17.1	6.3
IS 18390	15.4	12.5	17.5	12.5
IS 18489	41.0	40.0	17.9	10.0
IS 18367	52.5	50.0	20.0	12.5
IS 18479	70.3	62.5	21.6	18.8
IS 18319	70.7	100.0	24.6	18.6
IS 18387	87.5	100.0	25.0	20.0
IS 18349	57.5	100.0	25.6	28.3
IS 17775	100.0	100.0	27.5	20.0
Serena	100.0	100.0	31.6	17.3
CSH—1	100.0	100.0	33.3	27.1
IS 18328	75.6	100.0	34.1	39.4

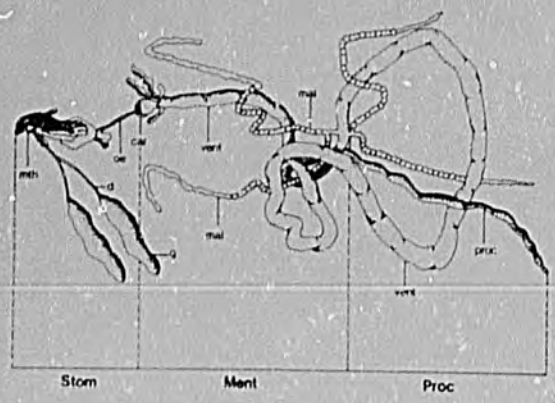


Fig 3: The alimentary canal of the third larvae instar of the sorghum shootfly, *A. soccata*

instar larva of sorghum shootfly forms a tube, variously looped upon itself; its total length exceeds 3.5—4 times that of the entire body. The tube shows only little differentiation beyond the primary division into short stomadaeum (Stom), long mesenteron (Ment) and proctodaeum (Proc.). The ventriculus takes the form of an elongate sac of approximately uniform diameter (vent.). The anterior end sac of the ventriculus is distinguished as a small circular — the cardia (car), (Fig. 3). The pair of salivary glands lie below the mesenteron of the alimentary canal.

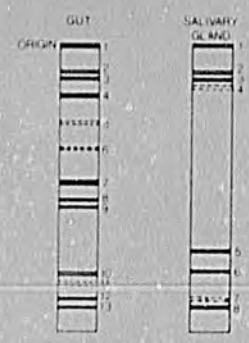
Thirteen fractions were obtained when electrophoresed extract of gut was stained with CBB-G (Fig. 4) Band nos. 1 and 3 stained intensely with CBB-G. Dotted lines (bands 5 and 6) indicate very lightly stained bands. The salivary gland homogenate could be separated into 8 protein bands. Again band nos. 1 and 7 stained intensely with CBB-G (Fig. 4a).

When the gels were stained for esterase activity, 6 bands, were seen for gut homogenate. These esterase bands appeared to correspond respectively to protein bands 1, 4, 7, 8 and 10. Two of the esterase bands from the salivary gland homogenate corresponded to protein bands 3 and 6 (Fig. 4b).

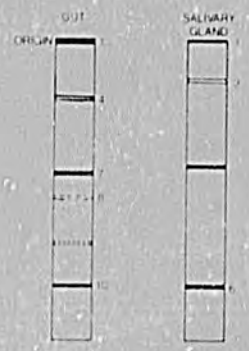
The electrophoretic mobilities of aminopeptidase bands from gut homogenates were similar to protein bands 13 and 7 (Fig. 4c). Aminopeptidase bands from salivary gland homogenate appeared to correspond to protein bands 7 and 8.

One amylase band was revealed from salivary gland homogenate, which corresponds to protein band no. 2. No amylase activity was observed in the gut preparation (Fig. 4d). Pepsinogen activity could not be detected in any of the gut or salivary gland preparation.

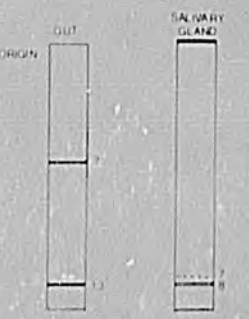
So far only 4 enzymes have been studied. We intend to study other enzymes, especially carbohydrases using fluorogenic substrates in the near future to identify the activity of the enzymes in the larva feeding on resistant and susceptible cultivar.



a. Electrophoretic protein pattern from the gut and salivary gland homogenates of Atherigona soccata



b. The banding pattern of esterase enzyme observed in the gut and salivary gland homogenates of Atherigona soccata



c. The banding pattern of aminopeptidase enzyme observed in the gut and salivary gland homogenates of Atherigona soccata

d. Amylase activity in the salivary gland homogenate of Atherigona soccata. No activity was observed in the gut preparation

Fig 4: Electrophoretic banding pattern of various enzymes in the gut and salivary gland homogenates of *A. soccata*

Experimental Bases of Insect Mass Rearing

R.S. Ochieng and F.O. Onyango

Screening of maize, sorghum, rice and cowpea cultivars for insect resistance requires the development of good techniques for mass production of insects at minimum cost and maximum reliability.

In view of the importance attached to research on cowpea resistance to the legume pod borer, *Maruca testulalis*, high priority was placed on the establishment of rearing procedures to ensure a continuous abundant supply of the pest. In 1980 efforts have therefore been concentrated on improving the rearing techniques developed during 1979. This technique is based on using flowers.

A procedure has been developed which allows production of 75,000 eggs of *Maruca testulalis* per month (Fig. 5). More than 170 eggs moth were obtained. Leaves with eggs were collected from young potted cowpea plants placed in oviposition cages and left for incubation in a petri-dish. The translucent eggs turn dark and hatch within 2 to 3 days. Fifty flowers, each containing a larva, were placed on nylon netting which had several holes and the top of the rearing box was securely covered with fine white cotton cloth. This provided adequate aeration, and eliminated accumulation of moisture in the box. The introduction of a greater number of larvae into the rearing box significantly reduced larval survival: at a density of 50 larvae per box survival was 80% diminishing to 41% at a density of 75; 37% at 100; 20% at 125 and 150, and 10% at a density of 200 larvae. fresh food was provided at two-day intervals. Larvae in the used food were lifted by holding the sides of the nylon mesh and placing them on top of the fresh food without touching them individually. There were 4 food changes, the last occurred on the eighth day, when larvae in their pre-pupal stage were transferred to the pupation medium.

Pupae were collected every 2 days by lifting the wire mesh from the sand and searching for the cocoons. After collection, the pupae were transferred singly into sterile glass vials for adult emergence. Thus, emerging adults could be easily sexed. Pupation lasted between 5 and 14 days, with a mean of 8.5±1.3.

The oviposition cage was a box 50 cm high, 35 cm wise and 45 cm long. The optimal number of moths placed in the mass oviposition cage with potted cowpea plants was 30. The average life span of the moths was 7.7 and 9.5 days per female and male respectively.

Rearing of insects on natural food materials has besides some advantages, also some limits. Therefore, experiments on artificial diets for *Maruca* rearing has been initiated under insectary conditions. So far fourteen different combinations of diets were tested. Different portions of leguminous grain powders (beans, cowpeas, soya) and dried parts of cowpea plants were incorporated into basic wheat germ diets used for rearing of lepidopterous species. The diets were dispensed in glass vials 2.5 cm x 7.5 cm and ten first instar larvae were placed into each vial. In the case of most of these diets, larval mortality was high. Only Kabali flour diet containing 5% of cowpea flower powder and wheat germ/soya composition was found to satisfy the gustatory and nutritional requirements of *Maruca* larvae.

Work on artificial diet for *Eldana saccharina*, an important pest of maize, sugar cane and sorghum in West Kenya was initiated in July 1980. Over 21 diet formulations were tried with various degrees of success. The fecundity of moth reared on the most suitable diet ranged from 131 to 236.

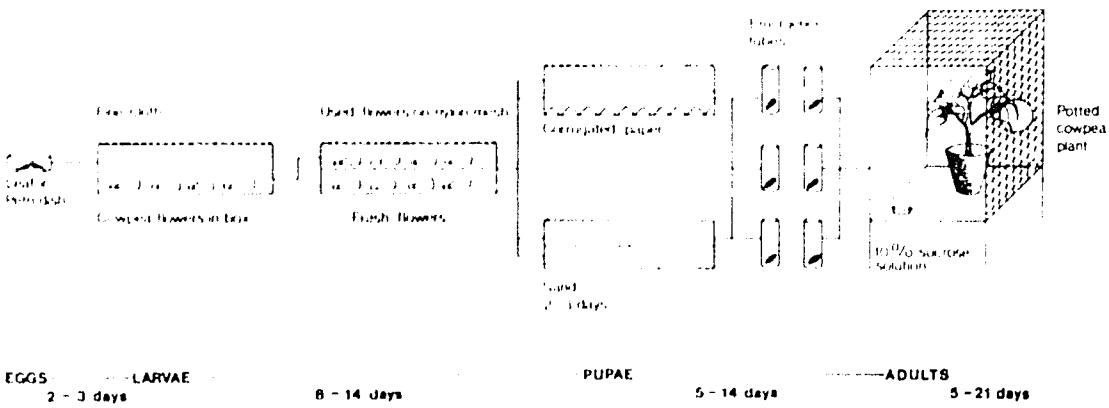


Fig. 5. Technique used in *Maruca testulalis* on natural diet.

Status of Pest Populations Under Mixed Cropping Systems

E.O. Omolo and K. Ogwaro

Intercropping is the traditional practice of subsi-

stence farmers of the tropics. While it is generally recognized that this system which evolved over extended periods of trial and error provides some advantages, little research has been directed to the resulting ecological relationships and the ensuing possibilities for pest management. Interplanting usually involves a tall crop, such as corn, sorghum and a short one such as cowpea, peanuts and beans. This system may reduce insect infestation because the non-host crop may interfere with the pest's ability to find its host, and the tall crop may obstruct the view of pests of the short crop. Maize, sorghum and cowpea lines showing moderate resistance to one or few major pest species under monocropping conditions may change their response to the target species or may react differently to other insect species under intercropping conditions.

The plot size was 100 sq. m. which is considered the minimum practicable plot size taking into account edge effects. The relationship between maize and sorghum was at a 1:1 ratio, and the spacing between rows was 90 cm and between plants 30 cm. Yields and yield components were observed and reported on a farm basis but for the final report will be combined for all locations and years. Yields of different crops are not comparable as individual crop components in the mixture possess different yield potentials and values. Thus it was advisable to report yields in equivalent land area. This was calculated for each intercrop plot, using pure stand data from the same block with the values statistically analysed. A value of <1 indicates that intercropping was less productive than pure stand.

$$\text{Equivalent area} = \frac{\text{Yield of maize intercropped}}{\text{Yield of Pure stand maize}} + \frac{\text{Yield of Sorghum intercropped}}{\text{Yield of Pure stand Sorghum}}$$

It is worth noting that, where maize, sorghum and cowpea were mixed, the main crop was maize and the other two were minor crops normally filled in at the farmers convenience.

Significant lower sorghum infestation by the shootfly was observed on plots with maize-sorghum than on pure sorghum plots (Tab. 7). Combinations of maize and sorghum could be an appropriate mixture for controlling *A. soccata*. The next step is to find the optimum mixture. Stem-borers showed more preference for sorghum than for maize, but where sorghum and maize were planted together the population of stem borers increased (Tab. 7).

Cowpea had no influence on maize yield whereas maize had a severe influence on cowpea yield. This means that, crop management for interplanted maize should not be different from that of pure maize, but in the case of cowpea, interplanted cowpea requires a different management from pure stand cowpea. This forms the basis for the determination of management optima for intercropping.

Cultural Practices

The recommended mixture for inter-cropping is between a legume and a cereal and never between a cereal and a cereal, however, farmers in the area have always grown maize together with sorghum. Before this common cultural practice is challenged, scientific explanations have to be provided by the appropriate crop mixture trial in the farmers' fields.

Whatever combination is found appropriate will be tried in an extensive farmers' field trials before the practice is recommended to the farmers through respective national programmes.

Once the appropriate crop combination is established then studies on the following cultural practices will be undertaken:

1) Suitable varieties — resistant or tolerant varieties
2) Adaptability
3) Time of planting
4) Weed control
5) Rate of fertilizer
6) Ideal population
7) Harvesting period
8) Processing and storage

Table 7. Pest Status Under Different Cropping Patterns – Field Observations

	SORGHUM SHOOTFLY		CROP BORERS		POD BORERS		YIELD
	Eggs/ plant	% D.H.	No. of holes	No. of larvae pupae	% of infest	Others	Kg/plot
Maize			4.57	3.23			7.5
Sorghum	2.02	51.0	5.75	3.04			3.5
Cow Pea					57.0	8.0	1.7
Maize/Sorghum	1.79	26.0	5.34	3.38			5.7
Maize/Cowpea			4.77	2.67	13.0	3.0	6.9
Sorghum/Cowpea	2.06	40.0	5.03	2.87	37.0	7.0	2.8
Maize/Sorghum Cowpea	2.25	34.0	5.06	2.34	10.0	1.0	6.4
Mean	2.03	37.8	5.09	3.07	29.25	4.75	5.0
LSD .05	NS	*	NS	NS			.08*

All these will lead us into the development of a comprehensive pest management technique that will control pests under small holder farming system in the rural community.

ICIPE-IRRI RESEARCH PROJECT ON RICE BROWN PLANTHOPPER *NILAPARVATA LUGENS* STOL

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In 1980, we focused our attention on the morphological nature of BPH biotypes and established a taxonomic basis for their identification. This scheme not only upholds the concept of BPH biotypes found within the Philippines, but also makes it possible to discriminate them from those occurring in other geographical areas. It thus demonstrates that BPH has been undergoing a certain degree of sympatric and allopatric speciation, and paves the way for future cytogenetic and biochemical investigations.

Our studies of BPH migration in the Philippine archipelago have shown that long-distance BPH migration has been taking place within the tropics. This finding has an important bearing on the spread of BPH biotypes and BPH-borne virus diseases which threaten the stability of the modern rice varieties. Highlights of these and other findings are given below.

Morphological Variations Among BPH Biotypes

BPH biotypes occurring within the Philippines and other Asian countries pose a serious threat to the stability of resistant rice varieties presently under cultivation. While host-mediated behavioural and physiological differences have been well recognised among BPH biotypes, a taxonomic basis for their identification has not yet been established. We, therefore, made an in-depth evaluation of selected morphological characters, particularly those involved in chemoreception of Biotypes 1, 2 and 3, which have been maintained as stock cultures at IRRI for several years.

Multiple discriminant analysis using Wilks' Lambda method indicated distinct segregation of these biotypes. Rostral, antennal, and leg characters gave a significant degree of certainty in classifying the three biotypes. The predicted biotype composition of BPH populations formed separate clusters. Cytogenetic studies on above biotypes are in progress.

BPH populations from other rice growing countries in Asia are currently being examined for possible taxonomic differences.

Long Distance BPH Migration in the Philippines

Studies of BPH migration in the Philippines initiated in the 1979 wet season were continued in 1980. Catches of delphacids were low on January 26 to February 2, 1980 voyage which was characterized by the prevalence of the Northeast Monsoon (20.5 – 32.7°C; 46 – 96% R.H.). No delphacids were caught on May 7 to 15, 1980 voyage which was conducted along Manila–Iligan–Manila route during the dry season 24.0 – 35.1°C; 43 – 92% R.H.). However, catches of delphacids, including BPH were quite high on the same route during July 15 to 24, 1980 (23.4 – 32.8°C; 59 – 98% R.H.) and October 17 to 25, 1980 voyages during which the Southwest Monsoon prevailed. These observations confirmed our earlier findings that migration of hoppers in the Philippines occurs mostly during the wet season which is characterized by a warm and humid air mass that flows constantly towards the Philippines Island during this period.

BPH Flight Activity

Flight in BPH is responsible for its dispersal from one breeding site to another and involves the following three phases: (1) Take-off during which macropterous hoppers depart from nutritionally depleted breeding sites; (2) Maintenance flight, during which insects may actively fly or be passively transported across distances in time and space, and (3) Invasion flights, during which insects alight on verdant rice fields for colonization.

Take-off activity was monitored by continous hourly catches of macropterous hoppers for seven consecutive days in a partially hopperburned rice field near IRRI Farm. Upwind and downwind BPH catches were made using sweepnets 1 m above the rice canopy at 5 sampling sites, 20 sweeps/site. Cumulative catches at each hour indicated a distinctly crepuscular, bimodal take-off activity. Males and females were caught in almost equal proportion. Light intensity of $\leq$ 10 lux ambient temperature of 21° – 25°C, R.H. of 80 – 100%, and weak wind of ca 1.2 KPH prevailed at around dawn and dusk, and seemed to favour BPH take-off. Sporadic hopper catches during the night may be attributed to the waxing moon phase which coincided with the observation period.

BPH invasion flights were monitored in IRRI fields planted with IR 1917 – 3 – 17, a BPH-susceptible selection, during two consecutive cropping seasons, December 1979 to March 1980 and May to August 1980, using yellow pan traps (YPT) and yellow board traps (YBT). Comparison of cumulative hourly BPH catches during both cropping seasons showed that the maximum number of BPH macropters was recorded at ca 0600 h in both types of traps, indicating a distinctly unimodal invasion flight activity. Light intensity of ca 100 lux and ca 6000 lux was

recorded at 0600 h during the first and second cropping seasons, respectively. Weak winds, relatively lower temperature and correspondingly higher relative humidity prevailed at the time of BPH invasion into rice fields than at any other time of the day.

BPH Resurgence Stone — Biochemical Basis

Application of certain insecticides, e.g. decamethrin and methyl parathion has been known to induce resurgence of BPH population in rice fields. However, the factors causing resurgence are not clearly understood. We investigated the biochemical changes taking place in the rice plant following the insecticide treatment. Results indicated that decamethrin, a strong BPH resurgence inducing insecticide, caused an apparent increase in total free amino nitrogen and a decrease in total carbohydrate level in leaf leaths of treated rice plants. Quantitative analysis of different amino acids is in progress.

Neem-Cake Blended Urea for BPH Control

Neem, *Azadirachta indica* (A. Juss.) has excellent insect repellent and antifeedant properties. Almost every part of the tree i.e. latter, bark, seed or seed kernel possesses maximum repellency. The neem cake, which comprises 70-90% of the crushed neem seed, is also a known nitrification inhibitor. These unique properties of neem cake prompted us to explore its potential for BPH control and augmenting rice yields.

Field trials in both dry and wet 1980 seasons showed that plots planted with IR17-3-3-17, a BPH susceptible selection, and treated with 20% neem cake + 120 kg N gave significantly higher yields than the control plots. Somewhat lower BPH nymphal populations were recorded in plots treated with 20% neem cake than in control. The incidence of virus diseases was markedly lower in the 20% neem cake treated plot.

Laboratory trials indicated that BPH population build up on IR8 rice plants treated with neem-cake blended urea was not as high as that in control pots treated with urea only.

Low cost of neem cake, relative safety to the environment, and its availability in many developing Asian countries, make it a valuable agricultural input which can easily be harnessed by an average farmer in Asia.

Effect of Neem Oil on Rice Leaf Folder (RLF)

Evaluation of neem seed oil in 1978 and 1979 against BPH and RLF *Chaphalocrocis medinalis* (Guenee), had indicated it to be an effective repellent, feeding deterrent, and oviposition and embryonic inhibitor. Further evaluation of neem oil against 5th instar RLF larvae showed that high developmental abnormalities and mortality were caused when the larvae were topically treated with neem oil or confined on treated rice leaves.

Efficacity of neem oil against other lepidopterous pests, e.g. the pyralid moth attacking *Azolla* fern, which is used for N fixation in rice fields, was also demonstrated.

Indigenous Plant Extract for Pest Control

The extract of an indigenous plant was highly effective, even in extremely small quantities, on BPH and several other insect pests and pathogens. It repelled insects, reduced feeding, disrupted embryonic and larval growth, and decreased survival and oviposition. The extract also inhibited growth of causal organisms of four bacterial and five fungal pathogens. It markedly inhibited the mycelial growth and spore germination of the rice blast fungus.

Analysis of the extract and identification of the active principles is in progress in collaboration with the Chemistry and Biochemistry Research Unit at the ICIPE.

CROP BORERS RESEARCH PROGRAMME

Programme Leader
Professor E.H. Smith (up to 28.2.80)
Research Advisor
Professor K.N. Saxena (1978)
Programme Coordinator
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Introduction

Crop Borers Research

A.K. Raina

The Crop Borers Research Programme was established in late 1979, with its main objectives as follows: (a) To identify the major borer pests of sorghum, maize, rice and cowpeas and assess the damage caused by each of the target species (b) study the ecology of these species under Mbita Point conditions (c) study the biology and reproductive physiology of selected pests (d) determine the insect plant relationships involved in traditional subsistence intercropping (e) collaborate with International Agricultural Research Centres and National Programmes and (f) establish relationship with subsistence farmers to serve as a two way bridge for the flow of information and testing of research findings. The purpose of all this is to develop environmentally safe and economically feasible integrated pest management practices.

The year 1980 saw two important events: An International Study Workshop on Biology and Control of the Sorghum Shootfly organized by ICIPE was held in Nairobi from May 5 - 8. In September the International Scientific Working Group on Cereal Stem Borers and Legume Pod Borers was held at Mbita Point Field Station. Both the Workshop and the working group produced vast information and helped shape the future of the Crop Borers Programme. Except for some specialized aspects, most of the Crop Borers Research was conducted at the Mbita Point Field Station where the hosts and their pests occur in their natural habitat. Many of the local farmers extended their cooperation in our research efforts by allowing us to conduct the experiments in their farms. Two more scientists and an agronomist joined the Programme. Work on rice stem borer was initiated at Mbita Point with great success.

The following report by participating scientists is a brief account of their significant findings during the year

Sorghum Resistance to Shootfly and Shootfly Behaviour

Ashok K. Raina

Sorghum Resistance to Shootfly

Based on the performance of the 1978 and 1979 international shootfly nurseries, 7 cultivars were selected for indepth investigation of the mechanism(s) of resistance. Experiments in the laboratory and greenhouse indicated that IS 2146, IS 3962 and IS 5613 were nonpreferred for oviposition in a single choice test. In a newly developed test using no choice with escape mechanism (Fig. 1), IS 1082 and IS 2312 were not preferred for ovipositon. These findings confirmed earlier observations in the field. IS 2146 caused high mortality and retarded growth among the larvae. Other cultivars that adversely affected the larval growth were IS 2312 and IS 3962. Four of these cultivars IS 1082, IS 2146, IS 3962 and IS 5613 along with the local variety 'Serena' were tested first in 1979 and again in 1980 at Mbita Point Field Station. Besides shootfly, they were rated for resistance to stem borers, midge and *Striga*, and for yield. IS 5613 performed very well for shootfly and midge. IS 2146 gave consistently highest yield. Incidence of *Striga* was lowest in Serena plots

Shootfly Behaviour

Oviposition marking, deterring pheromone

Having established the presence of an ovipo-

sition marking, deterring pheromone associated with the gene used by shootfly females to attach their eggs to sorghum leaves, work is in progress to identify the active compound. Various bioassay techniques were tried to deal with small quantities of fractions provided by the Chemistry Biochemistry Unit. The technique where the fraction is made into a solution with 0.01% Carbowax and applied with a cotton wafer to the leaves proved to be the most practical. Preliminary experiments indicate that the tarsi of the female are involved in detection of the pheromone.

Effect of manual dead hearts on infestation of tillers

It was reported earlier that the decaying shoots of sorghum (dead hearts) attract female shootflies and consequently increase infestation on tillers. Results of several replicated trials at NPES indicated that the presence of a dead heart does not influence infestation of tillers. A possible explanation is that although the dead hearts may attract flies for feedings, they do not elicit ovipositional responses. Consequently, the removal of dead-hearts will not reduce further infestation. However, by removing dead hearts, the potential source of the next generation of flies is also removed and this can be used as an effective method of shootfly control particularly in areas where farm labour is relatively cheap. It was observed that when the dead-hearts were pulled, the larva was removed with it 18% of the time. However, when the dead-hearts were cut at the base almost 70% of them had larvae. Four weekly removals of dead hearts from a 1 hectare sorghum field with an infestation of about 20% took 12 man hours.

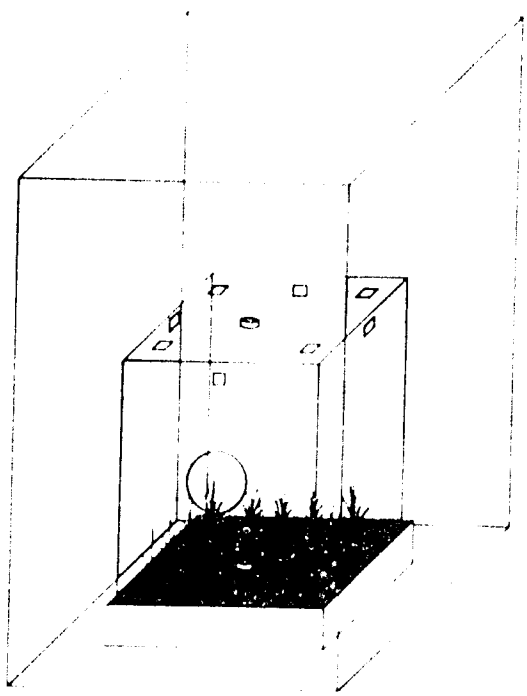

Fig. 1. [illegible] the oviposition preference of shootfly [illegible] sorghum cultivars by the method [illegible]

Some aspects of shootfly oviposition

The shootfly lays its eggs in the middle region of the abaxial surface of leaves of young sorghum plants. This location provides protection from being washed away by the rain. The female uses the tarsi in host selection, moving rapidly up and down the leaves of a plant. If acceptable the female then examines the oviposition site by extending its ovipositor and pressing it against the leaf surface. Later it grips the leaf edge with its tarsi and lays the egg, parallel to the leaf midrib. The actual act of oviposition takes about 40 sec. The female then clears its ovipositor and takes an flies away to another plant.

Distribution of eggs was studied in the greenhouse using CSH1 plants planted 20 tray. When the plants were in the 5 leaf stage, 2 pairs of flies were released over each tray in a cage. The flies prefer the 4th leaf followed by 5th and 3rd leaves (Fig. 2) less than 2% of the eggs were laid

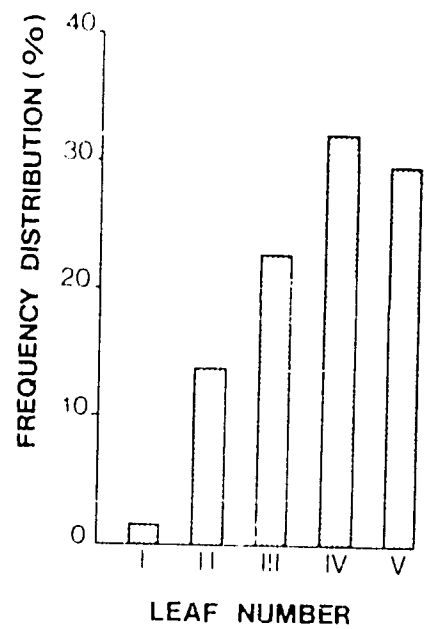

Fig. 2. Leaf [illegible] of oviposition, shootflies on [illegible] plant in the greenhouse.

on the 1st leaf. With an egg density of 1.2 eggs/plant the distribution of eggs followed the Poisson distribution (Fig. 3a). However, when the density increased to 2.2 eggs/plant, the χ^2 test indicated a significant deviation from Poisson distribution (Fig. 3b). The percentage of plants with more than 1 egg increased significantly. It was observed that once the fly ran out of choice plants for oviposition, further egg laying occurred next to previously laid eggs.

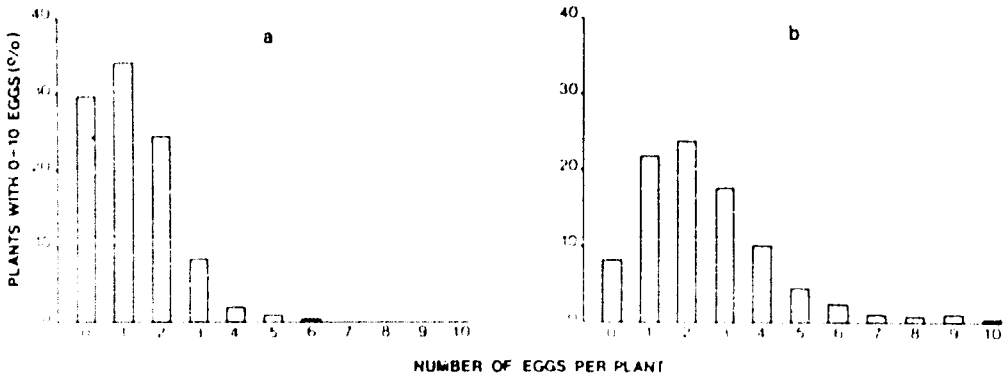

Fig. 3. Distribution of shootfly eggs on sorghum plants in greenhouse: a) with a density of 1.2 eggs/plant and b) 2.2 eggs/plant

Sorghum Shootfly Ecology

A.G.L. Delobel

Research initiated in 1979 on population dynamics of *A. soccata* was continued in 1980, chiefly in Western Kenya, with the aim of understanding the mechanisms underlying the seasonal fluctuations of the pest. It is generally assumed that, in Eastern Africa, the onset of rains induces a massive emergence of sorghum shootfly adults which are responsible for a sudden increase in sorghum infestation; some authors, however, have observed that populations reached their maximum, well after the onset of the rainy season, sometimes even during the dry season.

A series of monthly plantings was carried out at Mbita Point Field Station over a period of one year; observations indicated that *A. soccata* populations remained active on irrigated sorghum throughout the year; however, two peaks in adult and egg numbers occurred, a minor one in January, soon after the short rainy season and a major one in July – August, after the long rainy season. This last peak may be estimated to have started two months after the onset of rains and lasted until three months after the end of the season.

Several factors have been considered to find an explanation for these fluctuations. First, the host material is not present in equal quantities throughout the year in Mbita Point: sorghum is widely grown only once a year, during the long rains, the short rains being usually preferred by the farmers to grow maize. The peak observed in July – August at the field station therefore corresponds to a peak in the number of adults present in the area. Tiller development also shows a wide variation throughout the year; in particular, tillering is increased during periods of high rainfall, both because of accelerated growth and development of the plant and of increased shootfly infestation. The fact that, during the one year period covered by the survey, tillers did not receive significantly less eggs than the seedlings is noteworthy and indicates that tillering has certainly to be taken into account in population studies.

Partial life tables (excluding the adult stage) have been derived from field population observations and from separate mortality studies. Egg mortality shows little variation throughout the year, with a minimum of 9.4% in September 1979 and a maximum of 28.8% in April 1979; however, in a few instances when a heavy shower occurred within 48 hours after oviposition, a very high mortality (up to 75.6% in April 1980) was observed. Mortality is generally very high in the first larval instar; it is the result of competition for the available sorghum stems, specially in periods when the number of eggs largely exceeds the number of susceptible stems, as was the case in June to September 1980 when mortality reached 87%. This density-dependent mechanism caused a reduction in the populations before they could build up to large numbers. Under low population conditions, a certain degree of competition still exists because eggs are laid randomly among the plants, but mortality is usually reduced to less than 50%.

Mortality in the third instar was assessed for larvae which were artificially introduced in plants; it was found to be very high (between 53 and 88%), which is probably an overestimation. Mortality factors in this stage were the failure of the larva to re-establish itself in the stem, parasitization by *Tetrastichus* sp. (1.4 to 8.1%), diseases (3.1 to 11.6%) and failure to pupate. Pupal mortality varied between 9 and 100% and is clearly related to soil and plant humidity. The percentage of larvae pupating in the soil increases with increasing humidity, while the depth of pupation also increases, as indicated in the 1979 report; pupae present in the first centimeters of the soil are probably more susceptible than others to dessication and to predator attack.

The life tables indicate an accumulated mortality from egg to adult of 93.1% in May-June, 91.6% in June – July, 99.2% in July – August on seedlings, of 99.4% for the same period on tillers. The first budget corresponds to the end of the rainy season, at a period when the adult population in the area is still quite low; the second and third budgets correspond to a dry period

when adult populations are reaching their maximum, a heavy rainfall (49.2 mm) is responsible for a low level of infestation (85 eggs per 100 stems) of the seedlings in July August, whereas oviposition on tillers was not affected by rain and was found to be very high (230 eggs per 100 stems).

A general picture may be drawn from the series of observations made at Mbita Point during 1979 and 1980: the sorghum shootfly is present in the area, all the year round as active populations on cultivated sorghum (*Sorghum bicolor*) and on wild sorghum (*Sorghum arundinaceum*). Populations start increasing several weeks after the beginning of the rainy season, and the maximum is reached during the dry season. The sowing of sorghum by local farmers gives a sudden boost to sorghum shootfly populations, thus, sorghum fields planted towards the end of the rainy season are heavily infested. The most important mortality factor (density-dependent) is competition between first instar larvae for the available sorghum stems, which are susceptible only during a very short period. Another important mortality factor (density-independent) is heavy rainfall, which washes most of the eggs from the leaves and may also prevent the females from egg-laying.

Aspects of Reproduction in the Sorghum Shootfly

G. C. Unnithan

Influence of adult nutrition on oocyte development, oocyte resorption, and survival of the sorghum shootfly

The influence of adult nutrition on egg development, oocyte resorption and survival of adult shootflies was investigated. Shootfly females fed on the commonly used adult diet of 1:1 brewer's yeast and glucose had about 30% of their follicles degenerated before completing development during the first ovarian cycle. This process of oocyte degeneration is known as oocyte resorption or oosorption. In order to determine whether resorption is caused by a nutritional deficiency, the frequency of resorption in shootfly females fed on 11 different diets or their combinations was compared to that of flies collected from the field. Results are presented in Table 1. Resorption takes place only after vitellogenesis reaches a certain stage of development. In flies fed on water and protein alone vitellogenesis did not occur and hence there was no resorption. Honeydew from sorghum aphid appears to be the best of all the diets tested; frequency of resorption being only 13.6% although slightly higher than in flies collected from the field which had access to natural food.

Table 1

Influence of Nutrition on Oocyte Resorption in *A. soccata*

	Type of food	No. of insects**	Total No. of ovarioles	No. of ovarioles with resorbing oocytes (%) ***
1	Distilled water (H_2O)*		--	--
2	Baker's yeast + H_2O*			--
3.	Brewer's yeast + H_2O*		--	--
4	Casein + H_2O*	-	--	--
5	Glucose + H_2O	22	752	**37.89 a**
6.	Glucose + milk powder (1:1) H_2O	19	633	**37.28 a**
7	Honey + H_2O	21	722	**29.64 b**
8	Brewer's yeast + glucose (1:1) + H_2O	102	3583	**29.04 b**
9	Baker's yeast + glucose (1:1) + H_2O	18	598	**27.09 b**
10	Baker's yeast + sugar (1:1) + H_2O	62	2156	**19.99 c**
11.	Sorghum aphid honeydew + H_2O	84	2786	**13.64 d**
12	Natural food (flies from field)	26	852	**8.68 e**

* In these insects vitellogenesis did not take place, and most of them died within two days.

** Includes only those insects in which vitellogenesis has progressed to at least halfway through.

*** Figures followed by the same letter are not significantly different from each other, others significant 0.001 level.

Adult nutrition has considerable influence on the rate of vitellogenesis, duration of ovarian cycle and oviposition as evident from the data presented in Table 2. Length of the basal oocyte gives an indication of the state of development of the oocyte and the rate of vitellogenesis. (A freshly laid shootfly egg measures about 1.1 – 1.2mm in length). Vitellogenesis was very slow in flies fed on brewer's yeast and glucose and hence the ovarian cycle and preoviposition period were prolonged. Baker's yeast and sugar-fed flies showed enhancement of vitellogenesis and decrease in the duration of ovarian cycle. In flies fed on honeydew from sorghum aphid there was a further acceleration of vitellogenesis and a decrease in the duration of the ovarian cycle and preoviposition period.

Quality of adult food not only influences egg production but also the survival of the adults. Sorghum shootflies on a diet of protein and water alone neither produce eggs nor survive for more than two days. Carbohydrate is essential for both

Table 2 Effects of Nutrition on Egg Maturation and Oviposition in *A. soccata*

	Type of food	No. of insects	Age (hrs)	No. of insects with gravid ovaries (%)	Length of basal oocyte (mm $\bar{x} \pm$ S.D.)	No. of insects oviposited (%)
1	Brewer's yeast + glucose (1:1) + H_2O	31	36	0.0	0.53±0.31	0.0
2	Brewer's yeast + glucose (1:1) + H_2O	35	48	8.57	0.84±0.30	0.0
3	Baker's yeast + glucose (1:1) + H_2O	29	48	38.70	0.91±0.38	—*
4	Baker's yeast + sugar (1:1) + H_2O	36	48	69.40	1.18±0.34	—*
5	Sorghum aphid honeydew + H_2O	40	36	17.50	0.96±0.29	0.0
6	Sorghum aphid honeydew + H_2O	33	48	91.66	1.29±0.86	34.18

* No host plants were provided for these insects

survival of the adult as well as for egg production. Maturation of at least the first batch of eggs can be completed without protein feeding; thus *Atherigona soccata* is an autogenous species. However, supplementing carbohydrates with protein reduces the frequency of oocyte resorption, thereby increasing the number eggs which complete maturation. Nutritional deficiency leads to high incidence of oocyte resorption and perhaps non-functional ovarioles. Baker's yeast appears to be superior to brewer's yeast as an adult food. Sorghum aphid (cereal aphid) honeydew was more nutritious than all adult diets tested. The presence of the cereal aphid in the field may have a positive influence on the reproductive potential and therefore population build up of the sorghum shootfly. Studies are in progress to determine the effect of honeydew on fecundity of the shootfly.

Influence of mating: presence of males on oocyte maturation and resorption

Mating is known to influence egg maturation in several species of insects. Experiments were conducted to determine whether mating or the presence of males have any influence on egg maturation and resorption and oviposition in sorghum shootflies. During the first ovarian cycle neither egg maturation nor oocyte resorption was influenced by mating or by the presence of males. However, mating indirectly influenced maturation of eggs during the second and subsequent ovarian cycles as lack of mating delayed or prevented oviposition and consequently the development of the subterminal oocytes.

Effects of Precocene on sorghum shootfly adults, eggs and pupae

Effects of the anti-juvenile hormones Precocene I (PI) and Precocene II (PII) on adults, eggs and pupae of the sorghum shootfly were investigated. The toxic effects of PI and PII were reported in the 1979 ICIPE Annual Report. Further studies have shown that only 71% of 3-day-old females treated with 5ug of PII have gravid ovaries compared to 90% for females treated with PI and 100% for control insects. Frequency of oocyte resorption is also higher in PII treated females. The increased frequency of resorption and delay/inhibition of maturation of eggs appears to be due to the non-specific effects of PII. Exposure of gravid females to PI and PII resulted in inhibition of embryogenesis and hatching of eggs laid after treatment. Fumigation of eggs with PI inhibited hatching, but PII did not inhibit embryogenesis or hatching. Both PI and PII have no discernible effect on pupae when applied topically. In conclusion, there is no indication of PI and PII having any definite anti-juvenile hormone activity on sorghum shootflies. However, both compounds show very high toxicity and ovicidal effects.

Intensity Levels and the Effect on Yield of Stem-borers in Maize and Sorghum under Different Intercropping Patterns

Kennel Ogwaro

The spotted stem borer, *Chilo partellus* Swinhoe and the maize stem borer, *Busseola fusca* Fuller, are regarded as the most damaging borer pests of cereal crops in Africa. The sugarcane borer, *Eldana saccharina* Wlk is basically a pest of sugarcane in West Africa, but recently has been spreading into cereal crops throughout Africa.

Relative abundance of stem borer species

The results of observations on species distribution of stem borers at Mbita Point Field Station

have shown that out of 7678 borers collected from maize and sorghum, 92.7% were *C. partellus* 6.1% *E. saccharina* and 1.2% *B. fusca* on farmer's fields, 25 kilometers away. *E. saccharina* did not exist, and out of 3778 borers collected, 52.4% were *C. partellus* and 47.6% were *B. fusca*. *Chilo* appeared quite early in the growing season and continued to infest both crops throughout the season. *Busseola* and *Eldana* appeared later and their numbers reached maximum at harvest. Generally, there were fewer borers in the farmers' fields than on the experimental station.

Intensity levels and the influence of intercropping larvae in the vegetative stages of sorghum plants than of maize plants in pure stands, under intercropping maize had more borers than sorghum. However, during the late reproductive stage and until harvest time, sorghum was more infested in both intercropping and in pure stands. Although infestation levels remained lower in maize than in sorghum at harvest, intercropping increased the level considerably ($P < 0.001$), but had no influence on the number of borers recovered from sorghum (Table 3). Table 4 shows the mean number of borers per plant and the mean number of borers per internode bored, within groups of plants with different numbers of inter-

Table 3: Distribution of Stem Borers in Maize and Sorghum under Intercropping and Pure Crop Systems During Harvest

Cropping patterns	$\bar{x}$ No. of Plants/Sample	*Chilo partellus*	*Eldana saccharina*	*Busseola fusca*	Total	Mean No. of Borers/Plant
			A. MAIZE			
Intercropping	98 ± 1.2	136.5±47.5	55.0±45.3	3.3 ± 2.9	194.8±80.5	2.0±1.0
Pure Crop	99.5 ± 1.3	93.3±36.9	8.0±6.2	1.3 ± 1.3	102.5±35.3	1.1±0.4
	t = 96.46,	P<0.001				
			B. SORGHUM			
Intercropping	99.5 ± 1.3	533.3±55.7	57.0±21.6	7.3 ± 6.7	597.8±74.8	6.0±0.8
Pure Cropping	98.3 ± 1.7	530.3±154.8	61.2±37.2	7.3 ± 2.2	598.8±18.9	6.4±2.0
	t = 0.2, N.S.					

Plot replicates = 4

Table 4: Intensity Levels of Stem Borer Infestation in Maize and Sorghum under Intercropping and Pure Crop Systems at Harvest

		Number of internatodes per plant				Significance
			A. MAIZE			
$\bar{x}$ No. of borers per plant	P 0.5 ± 0.2	0.8	0.8 ± 0.2	1.2 ± 0.4	0.6 ± 0.8	t = 4.98
	I 2.1 ± 1.7	1.7 ± 1	2.0 ± 0.7	2.0 ± 1.2	2.9 ± 1.6	P≤0.02
No. of borers per internode bored	P 0.5	0.6	0.7	0.9	0.4	t = 3.76
	I 1.4	1.1	1.1	1.0	1.2	P≤0.05
			B. SORGHUM			
$\bar{x}$ No. of borers per plant	P 6.9 ± 5.3	5.9 ± 1.3	5.8 ± 2.4	4.9 ± 1.7	5.5 ± 2	t = 0.18
	I 4.0 ± 0.7	4.7 ± 1.4	5.1 ± 0.9	7.2 ± 0.6	9.1 ± 2.5	NS
No. of borers per internode bored	P 2.1	1.8	1.6	1.3	1.4	t = 0.65
	I 2.2	1.7	1.4	1.6	1.6	NS

P = pure crop I = intercropping

Intercropping maize and sorghum had a very significant effect on the distribution of the borers in the two crops, both at the experimental station and on farmers' fields. While there were more nodes. These means are significantly different under intercropping and in pure stands of maize (Table 4A).

Relationship between plant height and borer infestation

In the maize variety (Katumani) and sorghum variety (Serena) used in this study, the number of internodes per plant is a good indicator of plant height (the more internodes per plant the taller the plant). Figure 4 shows mean percentage internodes bored within groups of plants with different numbers of internodes. In maize crops there was a negative correlation between the number of internodes per plant and the percentage bored. Within all the groups of plants, the internodes bored were approximately 10% more under intercropping than in pure stand ($Y = 12.4 - 1.7x$; $r^2 = 0.76$ and $Y = 33.6 - 1.98x$; $r^2 = 0.98$) (Fig. 4A). In sorghum the relationship was positive under intercropping ($Y = 5.8 + 6.43x$; $r^2 = 0.98$), suggesting selection of taller plants, and negative in pure stands ($Y = 89 - 5.2x$; $r^2 = 1$), suggesting selection of shorter plants (Fig. 4B).

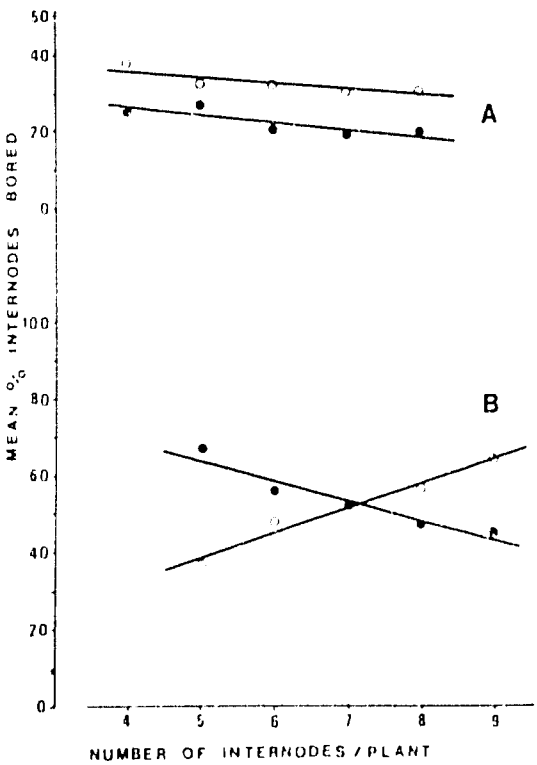

Fig 4: Correlation between the number of internodes per plant (plant height) and the mean % internodes bored o — intercropping; ● — pure crop. A — maize; B — sorghum.

Relationship between plant height and yield

Before attempting to correlate yield loss to insect infestation, the mean yields of plants of different heights were compared (Fig 5). It was shown that taller plants generally produced greater

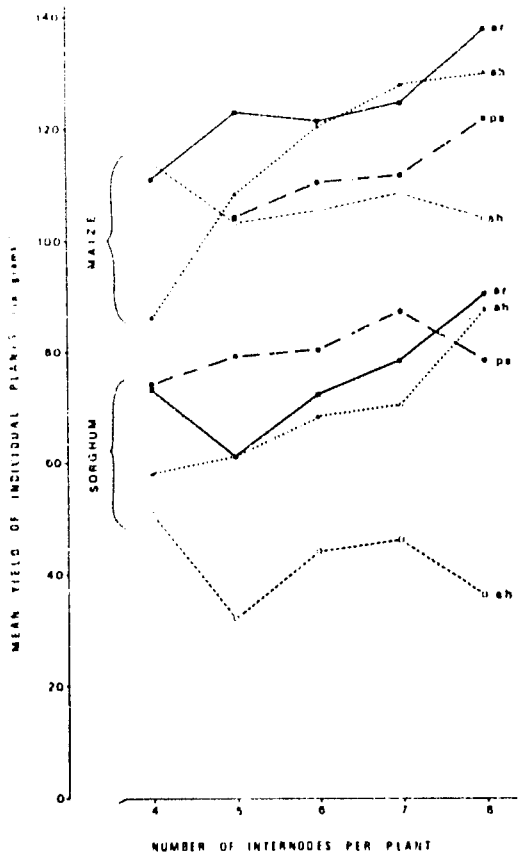

Fig 5: Mean yield of individual plants of different heights under different cropping patterns, ar, alternate rows; ah, alternate hills; ps, pure stand and sh, same hill

yield. However, the yield potentials were modified by, among other factors, the cropping patterns. Planting maize and sorghum in the same hole was disadvantageous to both crops, causing reduction in height and hence yield. However, alternate hill and alternate row planting increased the yield of maize due to an increase in area caused by shorter sorghum plants (maize grows faster than sorghum and therefore has an advantage).

Relationship between borer infestation and crop yield loss

When the relationship between % mean number of internodes bored per plant and crop yield was observed, it became clear that the borers had a considerable effect on yield, especially of maize. There was a negative correlation between the % mean number of internodes bored per plant and the maize seed weight (Fig. 6A). There was also a negative correlation between the % mean number

of internodes bored per plant and cob weight (Fig. 6). Complete loss in seed yield and in cob reproduction resulted at 35% of internodes bored damage in pure crop stands. However, under intercropping 52% of the internodes must be bored before complete loss in yield in seed and cob production results (see Fig. 6A and B).

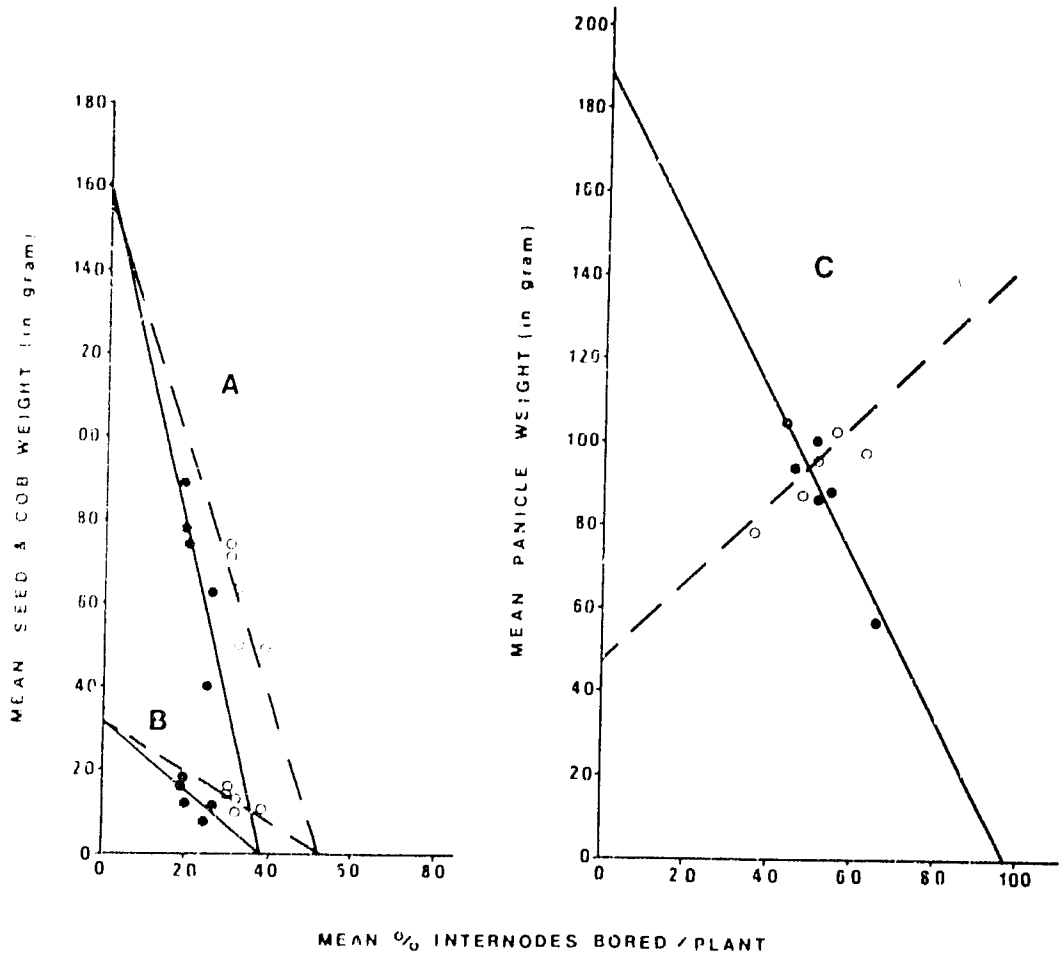

Fig 6: Relationship between borer infestation and crop yield loss. A = maize seed weight; B = maize cob weight. C = Sorghum panicle weight (O = intercropping; ● = pure crop

It is apparent from these observations that under intercropping, the borers either selected bigger and higher-yielding plants, therefore a higher % of damage was required to reduce the yield per plant under intercropping than in pure crop stands, or the maize plants were bigger under intercropping and could stand more borers than in pure crop stand. In sorghum the former is more likely to be the case. There was no effect of borer damage on the panicle weight under intercropping (Fig. 6). On the contrary, there was a positive correlation between the % of internodes bored and panicle weight. It was also shown that a much higher % of plants must be bored (97%) before complete loss in yield occurred in sorghum in pure stands (see Fig. 6).

Studies on Cowpea Pod Borer

J.B. Okeyo-Owuor

Survey for cowpea pests and status of *Maruca testulalis* (Geyer)

Field Surveys in South Nyanza during the long and short rainy seasons have revealed that cowpea is mainly grown in Ndhiwa, Kendu, Central Mbita and parts of Migori divisions. Ndhiwa is the only region where the crop is grown exclusively in the short rainy season. The pod borer *M. testulalis* occurs in all these locations and is the most important post-flowering stage pest. The flower thrip *Megalurothrip sjotedti* is also widely distributed.

Other pod borers like *Euchrysops* sp., *Lampides boeticus* L., *Cydia ptychora* Meyr. *Heliothis armigera* and some Agromyzid flies were relatively less abudant. Damage due to pod borers on cowpea flowers and pod was quite variable in various study areas. Fig. 7 shows the status of *M. testulalis* and other pod borers based on flower damage in 4 locations of Ndhiwa division during the short rainy season of 1980. At Mbita Point Field Station flower and pod damage due to these pests was 4.50% and 8.60% respectively. Damage in Lambwe valley plots was much higher (35 – 85% on flowers and 70 – 100% on pods). The lower infestation rate at Mbita Point Field Station could possibly be due to high mortality of *M. testulalis* larvae and pupae caused by disease pathogens and natural enemies. In both locations *M. testulalis* population was found to be significantly lower during the short rainy season compared to the long rainy season.

High infestation in some experimental plots where cowpea was not grown before and no other grain legume could be seen in the vicinity suggests the importance of alternate hosts since aestivation/diapause in *M. testulalis* has not been observed or reported. It is important therefore to search for and identify the role of such alternate hosts.

Resting and oviposition behaviour of the adult

Resting and oviposition behaviour of the moths was studied in 140 x 140 x 110 cm screen cages with potted cowpea plants and 50 pairs of adults/cage. *M. testulalis* is nocturnal in habit. During day time the majority of the moths rest on the lower side of the leaves (Table 5). Eggs were laid on all the above ground parts of cowpea plants with over 75% being laid on the leaves. The leaves therefore provide a suitable place for resting as well – oviposition for the moths.

the 1st and 3rd instar larvae. Soon after hatching the 1st instar larva vigorously searches for suitable feeding sites, which in this case are lateral and terminal buds and flowers. If artificially placed on leaves or stem, they quickly move to the tender parts of the plant and resume feeding. The 3rd instar larvae move to fresh flowers and tender pods. The 4th and 5th instars did not move much and fed inside the pods. They moved to adjacent pods if the food got exhausted or the pod became unsuitable due to excessive larval frass or mould development.

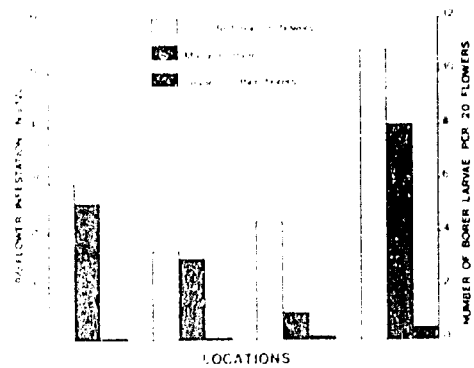

Fig. 7: The status of *M. testulalis* and other pod borers based on flower damage.

Table 5: Resting sites of *M. testulalis* adults during the day time

Time of observation (hrs)	% adults resting on				
	Stems	Leaves		Flowers	Pods
		Upper Surface	Lower Surface		
0700	2.1	8.2	75.4	12.2	2.1
1200	2.4	9.6	83.2	3.6	1.2
1800	4.9	8.1	85.8	0.0	1.2
Mean %	3.1	8.6	81.5	5.3	1.5

Larval movement and feeding behaviour

M. testulalis larvae have a high food searching capability. Greatest movement is exhibited by

In laboratory rearing experiments, movement from old diet to fresh diet was highest if the new diet was supplied after 72 hrs. (Fig. 8). Due to such movement behaviour, a larva could damage

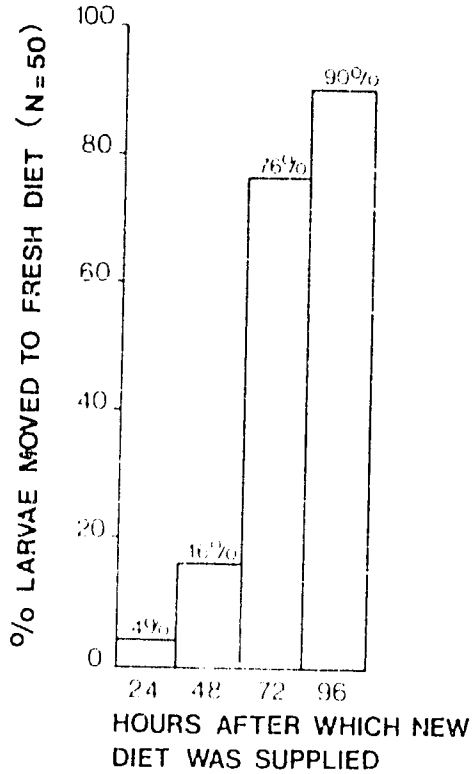

Fig. 8 Movement of larvae from old to fresh diet

several flowers and pods of cowpea throughout its 8–14 day feeding period. In cases where various plant parts touched each other the movement and subsequent damage was higher.

Rice Research at Mbita Point Field Station

J.J. Njokah

Currently 3 rice cultivars are grown in paddy plots at Mbita Point Field Station. These are 'Sindano' (a tall growing local variety), IR 579–48–1 and IR 1561–228–3–3 (semidwarf, high tillering lines of International Rice Research Institute origin). Efforts are underway to acquire more cultivars.

Pests encountered on rice with special emphasis on *Maliarpha*

The following stem borers have been recovered from the rice planted at Mbita Point Field Station: *Maliarpha* sp., *Sesamia* sp. and *Chilo* sp., of these *Maliarpha* seems to be the most abundant, followed by *Sesamia*. Infestation with *Maliarpha* starts during the late vegetative stage and reach a peak at the ripening stage (Table 6). Egg masses were found between folded leaf blades. During the ripening stage larvae and pupae were found in the hollow basal portion of the culm. The early instars of the larvae feed upwards within the hollow portion of the culm, causing the whiteheads. Before pupation the larva moves down, makes a hole in the side of the stem and pupates. Other pests recorded on rice were the whorl maggot, *Hydrellia* sp., the stalk-eyed fly *Diopsis thoracica*, the leaf roller and the mole cricket.

Table 6 Infestation levels of the rice stem borer *Maliarpha* sp. at 4 growth stages of 3 cultivars of rice at Mbita Point Field Station 1980

Cultivars	Growth State	Total No. of tillers/10 hills	No. of infested tillers	% infestation
	early vegetative	87	0	0.0
Sindano	late vegetative	194	5	2.6
	Reproductive	193	55	28.5
	Ripening	150	108	72.0
	early vegetative	152	0	0.0
IR 579	late vegetative	184	7	3.8
	Reproductive	201	9	4.5
	Ripening	294	72	24.5
	early vegetative	75	0	0.0
IR 1561	late vegetative	157	9	5.7
	Reproductive	186	11	5.9
	Ripening	275	62	22.5

Predation of *Maturpha* larvae and pupae: A reddish coloured ant was found feeding upon larvae and pupae of *Maturpha* at the base of the culm. It appears that the ants enter through adult emergence holes described earlier. These ants even feed on larvae in stubbles after the harvest and could contribute to substantial decrease in *Maturpha* population

AFRICAN ARMYWORM RESEARCH PROGRAMME

Honorary Programme Leader

Dr. D.J.W. Rose (1977)

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Dr. B.I.P. Persson (1977 — 1980) Research Scientist
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Armyworm Research Programme

D.J.W. Rose

Introduction

The financial grants which supported the armyworm programme came to an end in June 1980; and as a consequence the research was severely curtailed with the departures during the year of Dr. B. Persson and Dr. S. Khasimuddin. Both scientists had made major contributions towards understanding armyworm biology, and before they left all their work had been presented in manuscripts likely to be accepted for publication in international journals. Three papers by Dr. Persson are concerned with caterpillar behaviour in the African armyworm, population fluctuations in out-door cages and caterpillar mortality in relationship to colouration. His work has led to a better understanding of field mortality factors and possible causes of major outbreak years. It suggests that in-depth studies on the ecology of armyworm virus diseases may be fruitful. Dr. Khasimuddin has seven papers published or in press and two papers in manuscript which bring together his recent studies on diapause and caterpillar phase. Dr. Khasimuddin had concentrated in recent years on the problem of survival of armyworm during periods of the year when outbreaks were not known to occur. He followed up his discovery of diapause in the pupal stage and concluded that this is facultative in nature and seemingly rare in occurrence; and he was gathering evidence that caterpillars in the solitary phase are better adapted to survival.

The reports of the other members of the programme are given below. The techniques for determining the age of female moths, which are being studied by Bernard Otindo, are being used in field investigations and they are helping our understanding of short and long distance flights of moths. Jacob Yarro has nearly completed his studies on food plants and phenotypical variation and his research over the last three years is being presented as contribution towards gaining a Ph.D. degree at the University of Dar-es-Salaam.

Effects of Different Temperature Regimes on Oviposition

S. Khasimuddin

Larval infestations resulting in outbreaks of the insect occur at habitats that vary considerably in terms of temperature (altitude) and other factors. Forecasting of infestations and to some extent back-tracking of infestations does take into account the time of oviposition, egg hatch etc. It was therefore essential to get information on these attributes in relation to different temperature regimes.

Techniques and Methods

It is known that optimum oviposition occurs at 25°C ± 2°C. Therefore, three different temperatures were tried for attributes of age of first oviposition, female longevity, total oviposition and duration of egg hatchability. Constant temperature cabinets were used with constant humidity maintained at 75% ± 5% R.H. A 12:12 Light (L) dark (D) cycle was maintained.

The attributes were tested under two sets of conditions, one where single pairs were put under the temperatures immediately after emergence (unmated) and a second where single pairs were allowed to mate (2 days) under 25° ± 2°C and then put under the test temperature conditions (mated). The setup was similar to the one described earlier and food was provided in the form of 10% sugar solution.

Results and Conclusions

Results from these experiments are presented in Tables 1 and 2. It is seen that no significant difference occurs at the tried temperatures in as far as the age of females at first oviposition is concerned, whether they are subjected to these temperatures at emergence or after mating at optimal temperatures. Female longevity differs significantly with change in temperatures. When females are unmated and subjected to low temperature (12°C) they can live for up to four weeks. At 15°C and 18°C mating does not seem to affect their longevity. Females oviposit for up to 2 weeks under 15°C or 18°C which is an important information in terms of back-tracking and forecasting of infestations as these temperatures

Table 1. Ovipositional attributes of females of *S. exempta* under different temperatures – age at first oviposition, longevity and egg hatching

TEMPERATURE	Age at first oviposition (days)		Female longevity (days)		Time required for egg hatching (days)
	Unmated (X ± S.E.)	Mated (X ± S.E.)	Unmated (X ± S.E.)	Mated (X ± S.E.)	Unmated (X ± S.E.)
12°C	– (n = 26)	5.67 ± 1.12 (a) (n = 26)	28.73 ± 1.73 (a) (n = 26)	19.78 ± 1.46 (a) (n = 26)	No hatch
15°C	5.50 ± 0.56 (a) (n = 23)	5.63 ± 0.62 (a) (n = 33)	14.69 ± 0.65 (b) (n = 23)	16.17 ± 0.82 (b) (n = 33)	11.80 ± 0.09 (a) (n = 194)
18°C	4.56 ± 0.29 (a) (n = 16)	14.50 ± 0.73 (b) (n = 11)	13.82 ± 1.21 (c) (n = 16)	7.11 ± 1.24 (c) (n = 11)	7.11 ± 0.17 (b) (n = 63)

Similar letters denote no significant difference and different letters denote significant difference. Level of significant varies from P<0.05 to P<0.001

Table 2. Ovipositional attributes of females of *S. exempta* under different temperatures – duration of oviposition and number of ovipositions

TEMPERATURE	Total duration of oviposition (days)		Total number of ovipositions	
12°C		3.94 ± 1.41 (a) (n = 26)	– (n = 26)	1.78 ± 0.33 (a) (n = 26)
15°C	6.56 ± 0.69 (a) (n = 33)	7.43 ± 3.77 (b) (n = 33)	4.90 ± 0.46 (a) (n = 23)	5.15 ± 0.48 (b) (n = 33)
18°C	4.75 ± 0.47 (a) (n = 16)	4.0 ± 0.78 (a) (n = 11)	3.94 ± 0.41 (a) (n = 16)	2.91 ± 0.49 (c) (n = 11)

Within columns similar letters denote no significant difference and different letters denote significant difference – Level of significance varies from P<0.05 to P<0.001

are not uncommon in many habitats of the insect during a normal outbreak season. Hatching of eggs is affected by change in temperatures. At 12°C eggs do not hatch while at 15°C and 18°C hatching occurs after 11.8 and 7.1 days respectively. This is again significant in terms of back-tracking and forecasting. Normally 3 – 4 days time is considered normal in back-tracking of infestation. The results presented here suggest that the practice needs to be modified to take into account the prevailing temperature conditions at places of larval infestations.

The duration of oviposition (Table 2) does not seem to differ whether the females are subjected to these temperatures on emergence or after mating under optimal temperatures. At 15°C the females seem to oviposit for a longer time as well as oviposit significantly more times as at 18°C. The total number of ovipositions by a female is not affected by the temperatures if the female is subjected to these temperatures from emergence time. However, if a female mates at optimal temperatures and is then subjected to these temperatures, she will oviposit for a significantly greater number of times at 15°C than at 18°C.

The most important attribute from these results seems to be that at 12°C if emergence occurs, no mating and oviposition can occur, while at 15°C and above oviposition can be expected. It therefore appears that the threshold temperature for egg-maturation, mating, and oviposition lies between 12°C and 15°C. Earlier work on threshold temperatures for flight (Aidley, 1974) indicated 13.4°C as the threshold for flight initiation and it seems reasonable to suggest that the threshold temperature for egg-maturation, mating and oviposition is probably similar. Experiments to determine this could not be carried out due to lack of time.

The lack of oviposition of "unmated" females at 12°C suggests that egg-maturation is affected, as pre-mated females do oviposit at this tempera-

ture but their number of oviposition (total eggs laid) is significantly smaller. It is probably the eggs matured during the first 2 days at 25°C, that are deposited in this case, and no further oogenesis occurs.

Studies on the Comparisons of Haemolymph Proteins in Solitary vs Gregarious Larvae

S. Khasmuddin

As a further step towards understanding the physiological differences among the two categories of larvae, the haemolymph proteins of both the categories were determined. These include determinations of protein, glycoproteins, lipoproteins and glycolipids.

Methods and Techniques

The technique used was the disc gel electrophoresis. Haemolymph was collected from the larvae by excising one of the prolegs of the larvae and collecting the exuding haemolymph in micropippettes. About 5ul of haemolymph from each category of larvae was mixed with 20% sucrose and applied directly on the large pore gel. The electrophoresis was carried out in glass tubes with an internal diameter of 2.5 mm, with tris glycine buffer (pH 8.5) at 1.5 mA/gel for 35 minutes. Sample and spacer gels were prepared as described by Davies (1964), but the separation gel used here consisted of 7.5% cyanogum "41", 10 ammonium persulphate in tris-citrate buffer (pH 8.6) and N, N, N', N' tetramethyl ethylendiamine (TEMED). The gels were then stained for protein with Coomassie brilliant blue G(CBBG) (Diezel, et. al., 1972). Gels were also stained for glycoproteins using the PAS technique (Cross, 1975) and for lipoprotein using Sudan Black B (Maurer, 1971).

Results and Conclusions

The CBBG staining produced bands positive for proteins. These appeared as light to dark blue bands. The dark green haemolymph from gregarious larvae was separated into 21 protein fractions while the yellowish green haemolymph from solitary larvae was separated into 26 fractions, one intensely stained band appeared at the origin for both the categories. In the slow protein zone two wide and intensely stained bands were seen for gregarious as compared to only one for solitary larvae. These seem to be the major protein bands for both categories.

The major protein bands of gregarious larvae had however, a lower concentration of protein as shown by the width of the band. Further, it seems in gregarious larvae the major protein is composed of at least two fractions, but appeared as single protein, probably due to intense staining reaction. The remaining bands on both the categories stained moderately (CBBG) and were well separated.

Gels stained with PAS reagent produced 4 pink bands for gregarious and 5 for solitary. The four bands for gregarious corresponded to four protein bands indicating that these may be glycoprotein complexes. Similarly the five bands from solitary larvae also corresponded to protein bands again indicating glycoprotein complexes.

The Sudan Black B staining produced four bands for gregarious. Of these four, three bands corresponded to both protein and glycoprotein bands indicating these are glycolypids in nature. However, protein band No. 2 did not take the stain suggesting this as being glycoprotein. Similarly band No. 3 did not take the PAS stain, but stained with Sudan Black B indicating lipoprotein nature.

For solitary larvae four bands appeared positive with Sudan Black B suggesting glycolipid nature of these fractions. Band No. 3 did not take Sudan Black B stain suggesting its nature to be glycoprotein.

Table 3: Summary of electrophoretic analyses of proteins, glycoproteins, lipoproteins and glycolipids from haemolymph of solitary and gregarious larvae of *S. exempta*

	No. of fractions	
	GREGARIOUS	SOLITARY
Proteins	21	26
Glycoproteins	1	2
	(4)	(5)
Lipoproteins	1	—
	(4)	(4)
Glycolipids	3	4

Table 3 gives the overall picture of electrophoretic results from the two categories of larvae. It is seen that meaningful differences among the two categories of larvae exist for their total proteins, glycoproteins as well as glycolipids composition in their haemolymph. This forms an additional attribute of physiological differences between the two categories of larvae.

Moulting and Development of African Armyworm Larvae on various Host Plant Species

J.G. Yarro

Outbreaks of larvae of the African Armyworm *Spodoptera exempta* (Walk.) usually coincide with the germination of both wild and cultivated host plants. This coincidence of the occurrence of larvae with the abundant quantities of tender and nutritious food is the essence of the adaptation of

moths to the agency of winds that bring rains. It ensures fast and synchronous larval growth and development. The erratic weather conditions within the range of occurrence of the pest may result into discontinuation of good rains soon after the onset of the outbreak. Some outbreaks may occur towards the end of the rain season. In both circumstances, various grass species are likely to be too tough particularly to the early instars. This could also be looked at in the normal conditions, by studying the differences in development of larvae on various host plants which may differ in their texture.

In studying this problem first instar larvae obtained from parents maintained on maize leaves in an insectary at 25°C and 70% R.H were fed *ad libitum* on the following host plant species: star grass, *C. nodon dactylon* (L) Pers., maize, *Zea mays* L., Kikuyu grass, *Pennisetum clandestinum* Chiov. *Panicum maximum* Jacq. *Setaria plicatilis* (Hochst.) Hack., and a sedge, *Cyperus marangiensis* K. Schum. They were maintained at 18°C and 80% R.H., 25°C and 70% R.H. and 30°C and 60% R.H. The sedge was not included at 25°C and 70% R.H.

Samples of thirty larvae of each instar were taken from each of the host plants preserved in 70% ethanol and kept for measurements.

A further collection was obtained form outbreak sites at Athi River and Lukenya on *Cynodon dactylon* and *Pennisetum maximum* respectively.

Head capsule widths and distances between the frontal setae of the clypeus of the head capsule were measured using a dissecting microscope equipped with a micrometric scale. The regression of the distance between the frontal clypeal setae on the headcapsule widths is highly significant ($P < .001$ and $r^2 = .993$). Therefore any of them can be used for this purpose so long as the head capsule is intact but when it splits after moulting the clypeus remains intact and the distance between the frontal setae becomes more reliable in distinguishing between instars.

The results showed that larvae of *S. exempta* go through five instars in favourable conditions but under harsh conditions they may go through six or seven instars. The headcapsule widths and the distance between the frontal setae (DFS) increase at a geometric rate in the successive larval instars. The proportions of each of these parameters which are accounted for by the instars (R^2) are quite high and hence the suitability of both parameters. The rate of increase between the instars is lowered with the increase in the number of instars (Tables 4 and 5).

Table 4

(a) The relationship between headcapsule widths and instars of larvae reared on various host plants

Temperature and humidity	Host plant	No. of instars	Regression (b)	R2	Headcapsule width in last instar (mm)
18°C 80% R.H	*C. dactylon*	6	.177	.991	2.597
	Z. mays	6	.177	.990	2.687
	P. clandestinum	6	.173	.987	2.520
	P. maximum	7	.142	.986	2.630
	C. marangiensis	7	.141	.991	2.677
	S. plicatilis	7	.139	.987	2.424
25°C 70% R.H.	*C. dactylon*	5	.218	.999	2.548
	Z. mays	5	.219	.991	2.485
	P. clandestinum	5	.213	.997	2.396
	P. maximum	6	.175	.994	2.563
	S. plicatilis	7	.139	.997	2.509
30°C 60% R.H	*C. dactylon*	5	.215	.996	2.448
	Z. mays	5	.216	.994	2.465
	P. clandestinum	5	.218	.996	2.468
	P. maximum	6	.179	.976	2.530
	C. marangiensis	6	.178	.969	2.467
	P. plicatilis	6	.170	.999	2.545

(b) The relationship between headcapsule and instars in groups which went through similar number of instars

Number of instars	Regression (b)	S. E.	R^2	Equation
5	.216	.0044	.995	$y = 2.335 + .216x$
6	.176	.0031	.986	$y = 2.397 + .176x$
7	.140	.0032	.990	$y = 2.445 + .140x$

Where y is the logarithm $_{(10)}$ of the headcapsule width (μ) and x is the larval instar.

Table 5:

(a) The relationship between instars and the distance between the frontal setae (DFS).

Temperature and humidity	Host plant	Number of instars	Regression (b)	R^2	DFS in last instar (mm)
18°C 80% R.H.	*C. dactylon*	6	.184	.977	.428
	Z. mais	6	.182	.980	.412
	P. clandestinum	6	.178	.977	.406
	P. maximum	7	.146	.962	.432
	C. mauritanicus	7	.147	.982	.410
	S. plicatilis	7	.149	.979	.403
25°C 70% R.H.	*C. dactylon*	5	.229	.990	.397
	Z. mais	5	.251	.989	.398
	P. clandestinum	5	.227	.985	.388
	P. maximum	6	.187	.996	.407
	S. plicatilis	7	.149	.997	.391
30°C 60% R.H.	*C. dactylon*	5	.228	.999	.415
	Z. mais	5	.221	.983	.410
	P. clandestinum	5	.221	.994	.388
	P. maximum	6	.184	.982	.416
	C. mauritanicus	6	.187	.963	.408
	S. plicatilis	6	.179	.986	.414

(b) The relationship between DFS and instars in groups which went through similar number of instars

Number of instars	Regression (b)	S.E.	R^2	Equation
5	.226	.0060	.990	$y = 1.500 + .226x$
6	.183	.0042	.980	$y = 1.561 + .183x$
7	.148	.0044	.980	$y = 1.598 + .148x$

When y is the logarithm (10) of the distance between the frontal setae (μ) and x is the larval instar.

Table 6 Head capsule and DFS frequencies for Athi River and Lukenya populations

Headcapsule width (mm)	Athi River	Lukenya	DFSx 026 (mm)	Athi River	Lukenya
2.95 – 3.00	7	1	1.95 – 2.00	2	
2.85 – 2.90	37	2	1.85 – 1.90	5	
2.75 – 2.80	61	22	1.75 – 1.80	17	3
2.65 – 2.70	73	36	1.65 – 1.70	67	10
2.55 – 2.60	75	102	1.55 – 1.60	95	43
2.45 – 2.50	56	100	1.45 – 1.50	123	83
2.35 – 2.40	25	46	1.35 – 1.40	35	80
2.25 – 2.30	10	43	1.25 – 1.30	10	73
2.15 – 2.20	4	43	1.15 – 1.20	10	78
2.05 – 2.10	5	34	1.05 – 1.10	7	58
1.95 – 2.00	7	47	.95 – 1.00	2	56
1.85 – 1.90	4	49	.85 – .90		26
1.75 – 1.80	2	35	.75 – .80		7
1.65 – 1.70	–	19	.65 – .70		–
1.55 – 1.60	–	12	.55 – .60		2
1.45 – 1.50	–	8			
1.35 – 1.40		3			
1.0		1			

Larvae reared on star grass, maize and kikuyu grass went through five instars at higher temperatures (25°C and 70% R.H. and 30°C and 60% R.H.) but underwent an extra moult at the lower temperature (18°C and 80% R.H.). Similarly the larvae on *S. plicatilis* went through seven instars at lower temperatures (18°C and 80% R.H. and 25°C and 70% R.H.) but went though six instars at the highest temperature (30°C and 60% R.H.). These observations suggest preference for higher temperatures which is related to the host plant species on which the insect is feeding. The larvae which go through extra instars tend to have larger head capsules although the resulting pupae remain

smaller than the pupae formed by larvae which went through five instars.

The habitats differ with respect to the diversity of the host species and plants belonging to the same host species differ in their physical and chemical properties due to their positions within the habitat. These variations coupled with other environmental conditions like temperature have an effect on the size of the moulting instar and the subsequent instar size as well as the number of instars. If an instar starts at a small size it may grow faster to catch with the normal larvae if the condition improves. If the conditions are constant then the small size may be compensated for by one or two supernumerary instars. The variation in habitats cause large variations in instar size as demonstrated by larval populations at Athi River and Lukenya (Table 6). Athi River had plenty of food while the grass at Lukenya was drying up. There was thus a better synchrony of larval growth at Athi River than at Lukenya.

Physiological age of *Spodoptera exempta* (wlk.). investigations on ovary development

B. L. Otindo

Preliminary studies made on determination of the age of *Spodoptera exempta* (Wlk.) moths have investigated criteria such as numbers of cuticular layers and changes in their internal reproductive and non-reproductive systems. The development of follicles provides valuable information about ageing female moths.

Female moths were dissected in physiological saline and observations were made on nurse cells and oocytes along the lengths of the ovarioles. At the time of moth emergence the nurse cells were undifferentiated and the ratio of body weight/forewing length was at its maximum (range 3.16 – 4.40 for 120 specimens reared at 25°C). The follicle development became rapid within the next 12 hours. Later on, the nurse cells became differentiated and occupied about half the volume of the follicle. There was no corresponding rapid change in the ratio body weight/forewing length.

A marked change in the ratio body weight/forewing length occurred at 24 hours from the time of moth emergence (range 2.39 – 3.21 in 120 specimens). It was also observed that the nurse cells occupy about a quarter of the volume of the follicle probably through resorption of nurse cells.

Information obtained from ovary dissections is being applied to moths collected in the field and used to determine ages of female moths collected in light traps.

LIVESTOCK TICKS RESEARCH PROGRAMME

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Introduction

Tick borne diseases and the debility caused by tick infestation are major inhibitory factors on livestock productivity all over the world, but particularly in the tropics in Africa while most of the tick borne diseases can be controlled by vaccination or chemotherapy, East Coast Fever transmitted by *Rhipicephalus appendiculatus* remains to be dealt with. For this reason the only presently available method to achieve control is the close interval application of acaricides to cattle in dips or sprays. This procedure has many disadvantages. The two most important being that, firstly, ticks quickly become resistant to available acaricides and the cost of producing new ones is becoming prohibitive. Secondly once embarked on a system of control using acaricides it must be continued with 100% efficiency. The entire cattle population so treated, never having been exposed to ticks or tick borne diseases will be completely susceptible to both the ticks and the diseases and if for any reason acaricide control breaks down catastrophes will occur.

When vaccine or curative drugs become available for E.C.F. and we are assured by our colleagues at ILRAD and the Veterinary Research Department of KARI that there is a distinct possibility that both might soon be available, then alternative methods for controlling ticks should be considered.

As a result of these considerations ICIPE has embarked on a programme to investigate the feasibility of using biological methods for tick control, concentrating initially on *R. appendiculatus*.

Two main approaches are being followed:

1. Cattle become resistant to ticks following exposure in a tick infested paddock or the artificial application of a limited number of adult ticks.

The nature of this resistance and its effects on field populations of ticks is being investigated.

2. The second approach depends on the observation that immunoglobulins ingested in the blood meal of ticks are transferred directly into the haemolymph. If target antigens can be identified in the tick and antibodies produced against them, they will then attack the target antigen when ingested by the feeding tick.

The effect of host resistance on tick population development

R.M. Newson, J.W. Chiera and M.P. Cunningham

Two generations of *Rhipicephalus appendiculatus* have now been completed in this experiment which began in 1979. Four predictions are being tested concerning the effects of cattle with naturally acquired resistance to tick infestation, compared with susceptible cattle, on tick population development.

Three double-fenced paddocks of 0.6ha on Veterinary Research Department land at the Kenya Agricultural Research Institute, Muguga, were seeded in October 1979 with unfed *R. appendiculatus* larvae (21 per m^2). Cattle were introduced 10 days later; two susceptibles were put into paddock 1 and remained there to let ticks feed and develop resistance. Two susceptibles were also put into paddock 2. These were changed for fresh susceptibles three times. Two cattle already shown to be strongly resistant to tick infestation were put into paddock 3. Ticks were monitored on the ground by blanket dragging and hosts by counting adults and nymphs on the ears, by monthly collections of adults from half the body, and by scaping all the larvae and nymphs from clipped sample areas (3cm x 10cm) on the face, neck and lower forelegs. Test feeds on 100 nymphs were done on cattle ears in the laboratory, before the cattle were exposed to field challenge, to assess their resistance.

The predictions being tested were that:

(a) There would be little or no production of nymphs or adults in paddock 3, but there would be good production in paddocks 1 and 2.

(b) The breeding success of any adults reared in paddock 3 would be lower than in

paddocks 1 and 2.

(c) Susceptible cattle would develop resistance from exposure to ticks.

(d) Replacing the hosts in paddock 2 with fresh susceptibles at intervals would lead to a rapid increase in tick numbers.

The experiment proceeded according to plan until June, 1980 when all the cattle were temporarily removed to the laboratory for testing. The next pair of cattle could not be introduced to paddock 2 until late August on account of quarantine regulations that came into force. By this time the grazing in paddocks 1 and 3 was nearly exhausted and in mid-September it was necessary to remove all the cattle. A final assessment of the tick populations started at the end of November with the introduction of three pairs of susceptible cattle for 9 days. Monitoring the ticks on the ground continued without interruption.

Altogether 2300 counts and samples were taken and more than 6500 ticks were measured. The results are still being analysed but none of the predictions has been disproved. The population changes are summarised in Table 1 ignoring the first generation of larvae, since they were the original introduction. Differences between populations 1 and 2 were not expected until mid — 1980 since the cattle had identical tick experience until those in paddock 2 were changed for the first time in March, at the time that the second generation of larvae emerged. Thereafter differences began to appear between the populations, and these have increased. After only three days exposure the adult ticks counted on the ears of the final test cattle averaged 113, 651 and 6 for paddocks 1 — 3 respectively.

The scuta of adults and nymphs were measured optically. In the first generation of nymphs, those from paddocks 1 and 2 were significantly bigger than those from paddock 3, but those from paddock 1 were not significantly different from controls fed as larvae on a susceptible rabbit. In the second generation, and a newly-emerging third generation, the nymphs from paddock 2 remained the biggest.

In the first adult generation, males and females did not differ significantly in scutal size between paddocks 1 and 2, although both were significantly ($P < 0.001$) bigger than from paddock 3. By the second generation the ticks from paddock 2 were significantly bigger than those from the others, which no longer differed from one another, as those in paddock 1 became significantly smaller.

Chiera et al (following report) showed that at 43% relative humidity and 28°C, survival of unfed adult ticks was correlated with weight in both sexes. Survival was also correlated with scutal length, since that correlates with weight. The scutal length differences observed in unfed ticks in the present experiment are directly interpretable as differences in potential survival time of about one third. Field conditions at Muguga are as shown above for only a few hours per day, but reduced survival must be a contributory factor in the observed changes in numbers.

In all cases the nymphal test feeds on the cattle before exposure gave results comparable to those from susceptible rabbits (i.e. more than 80% engorged with mean engorged weights greater than 8mg). After removal from the paddocks, and re-testing, both the percentage feeding and the engorged weights were roughly halved. When test feeds of adults and larvae were done, results similar to those reported in the Seventh Annual ICIPE Report were obtained. Larval feeding success was less than 10%. Although the majority of adults still fed, many detached prematurely and the mean engorged weight was reduced, resulting in egg productions of less than half the full potential.

Measurements were also made during these test feeds of the daily changes in length of individually marked females, in order to determine the size range the day before they detached. This information will enable us to re-analyse the field

Table 1. Summed collections of (a) Larvae and (b) Nymphs by blanket dragging from the vegetation, each input being the mean of 10 standardised runs on the same day. (c) Estimated total numbers of female ticks per host, derived from monthly half-body samples on both cattle in each paddock; n = number of sampling occasions

Generation	Instar	Period	n	Paddock No. 1	2	3
	(a)					
2	Larvae	Mar. 80—Jul. 80	9	378	441	17
3	Larvae	Aug. 80—Nov. 80	9	106	1294	11
	(b)					
1	Nymphs	Dec. 79—Jan. 80	9	9	4	2
2	Nymphs	May 80—Sep. 80	7	10	64	1
3	Nymphs	Oct. 80—Nov. 80	7	12	82	3
	(c)					
1	Females	Jan. 80—Jun. 80	6	347	489	31
2	Females	Sep. 80	1	130	802	23

samples to seek further evidence of host resistance effects in action, by comparing the proportions of late fed ticks versus early fed ticks in different situations.

Feeding and Breeding Performance of *Rhipicephalus appendiculatus* fed on tick resistant and susceptible hosts.

J.W. Chiera, R.M. Newson, M.P. Cunningham

We have shown (ICIPE Annual Report for 1979) that when nymphs attach on hosts resistant to tick infestation many fail to feed, or feed on less than their full potential. The weights of both sexes after moulting and, later, of the engorged females are related to the weight of the preceding engorged nymphal stage. Similarly, females fed on resistant hosts attain lower weights than those on susceptible, or tick naive, hosts. Their egg production is correlated with engorged weight. Positive linear correlations have now been obtained between the weight of the engorged larva and the weight of the moulted nymph (r 0.90), and between the weight of the engorged female and the weight of its egg batch (r 0.89) when fed on naive hosts.

In another experiment, 116 unfed adult *R. appendiculatus* were weighed individually. Their weights varied from 9.6–5.4mg depending on whether they had fed as nymphs on resistant or on naive hosts. Male and female ticks were allocated to 3 weight categories and fed on naive rabbits. They were offered normal colony ticks for mating partners. Normal colony ticks, mated with normal colony partners, served as controls. The engorged females were weighed. The eggs that they produced were also weighed and then assessed for hatchability. Male ticks of all size categories were comparable to normal colony males in their ability to fertilize females. However, females of the lowest size category (9.6 – 1.8mg), mostly failed to lay eggs, or if they laid eggs these did not hatch. The remaining females gave egg yields in proportion to their engorged weights. Thus ticks which fed on highly resistant hosts suffered an additional penalty, since many of them were unable to reproduce.

The rate of water loss/gain and survival of *R. appendiculatus* of differing sizes

J.W. Chiera

Poorly fed larvae and nymphs moult into undersized nymphs and adults, which have an increased surface: volume ratio. They are thus likely to lose water to the environment at a higher rate than normal. This could affect their capacity to survive until they find a host.

One hundred and twenty six unfed *R. appendiculatus* of differing sizes were individually weighed and allocated to three weight categories. The ticks were contained in nylon mesh bags in groups of threes. They were hydrated at 96% RH for one week, then kept at 43% RH and 28°C for four days. The bags of ticks were weighed daily. The changes in weight are shown in Fig. 1.

The smallest ticks lost and gained water much faster than the largest ones.

In another experiment, one group of 121 unfed male and female *R. appendiculatus* of various weights was held at 43% RH and 28°C whilst a second group of 99 ticks was placed at 85% RH and 28°C. The ticks were checked weekly for dead ones. After 8 weeks all ticks at 43% RH were dead. It can be seen under these extreme conditions, where the ticks had no opportunity to regain lost moisture that survival was related to weight (Fig. 2). The observations at 85% RH are not yet completed, after 21 weeks more than half of the ticks were dead, and a similar pattern of survival in relation to weight was again emerging. Results of this experiment confirm the supposition that a tick resistant host affects those ticks which succeed in feeding on it by impairing their survival whilst they await their next feed after moulting.

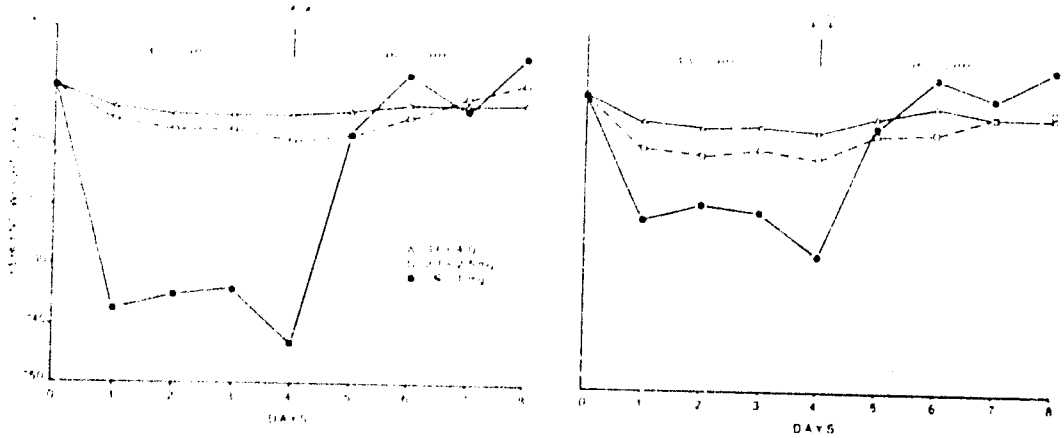


Fig 1. Weight changes of adult *R. appendiculatus* of differing sizes when exposed to 43% RH (Days 1–4) and 96% RH (Days 5–8) at 28°C

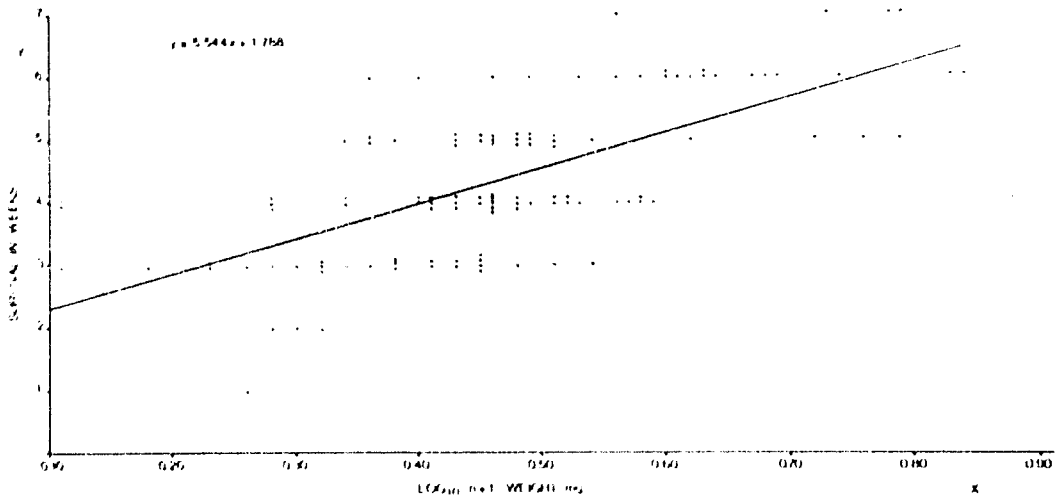


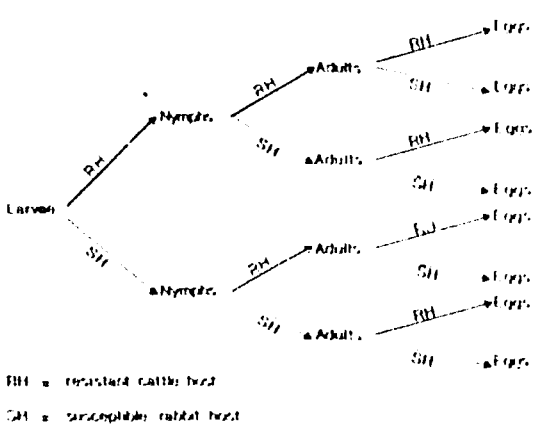
Fig 2: Survival in relation to weight in adult *R. appendiculatus* at 4 8% RH and 28°C.

The cumulative effects of host resistance on *R. appendiculatus*

J.W. Chiera, R.M. Newson, M.F. Cunningham

Rhipicephalus appendiculatus larvae, nymphs and adults were fed on all the eight possible combinations of resistant and naive hosts that a 3 host tick may encounter in nature. Cattle of varying degrees of tick resistance were used as the resistant hosts whilst, for convenience, rabbits were used as the naive hosts.

Rabbits are comparable to naive cattle in feeding *R. appendiculatus*. Batches of 6,000 – 12,000 larvae were applied in ear bags to resistant cattle and naive rabbits. The following feeding scheme was followed.



The percentage surviving each feed, the weights of the engorged ticks of each instar and the percentage moulting successfully were recorded. All engorged ticks and eggs were maintained at 85% RH and 28°C. The egg production of engorged females was weighed on the tenth day after the start of oviposition: any additional eggs were weighed on day 18. Taking the weight of a single egg as 0.04mg, the number of eggs produced per 1,000 original larvae was calculated.

Preliminary results using two pairs of cattle of varying degrees of resistance are given in Table 2. R67 and R68 were 2½ year old Zebu cattle from Mbita with 18 months of natural tick experience. They showed a steady level of resistance, and fed 6% and 11% of test larvae respectively. M207 and M211 had been exposed in a field experiment to larvae, nymphs and adults for 4 months prior to the present experiment. When the larvae were applied they were very resistant, allowing less than 1% to feed. However, when nymphs and adults were fed on them later their resistance

Host Combination	Resistant cattle host			
	R67	R68	M207	M211
RRR	28	6	0	0
RRS	35	31	0	0
RSR	22	56	0	0
RSS	107	193	0	0
SRR	227	108	65	334
SRS	705	214	413	550
SSR	337	480		559
SSS	1,299	1,282	1,071	1,052

Table 2: The Estimated number of eggs (in thousands) produced per 1,000 original larvae with various host combinations.

R = resistant host S = Susceptible host.
thus RRS = resistant host for larval feed, resistant host for nymphal feed and susceptible host for adult feed.

seemed to have waned. Reduced egg production has been observed whenever a resistant host is encountered in the life cycle, especially in relation to the feeding of the immatures. Reduced percentage feeding, reduced size of the ticks and reduced percentage moulting all reinforce one another to give a cumulative reduction in productivity.

Survival of *Rhipicephalus appendiculatus* in the field

D.K. Punyua

This long term experiment is a continuation of work reported in the ICIPE Seventh Annual Report (1979) and has now been running for 14 months.

Earlier reports indicated that some adult *R. appendiculatus* may survive in the field for about two years, but the immatures (larvae and nymphs) survive for a much shorter period. Since these early reports were based only on observations, a detailed study is now being made on survival (longevity), viability, and behaviour of *R. appendiculatus* in the field at the Veterinary Research Department, KARI, Muguga. Ticks from the laboratory colony at Muguga were released three times: In phase I in September 1979, phase II in February 1980, and phase III in July, 1980. Counted batches of engorged larvae and nymphs, almost at the point of moulting, and weighed eggs almost at the point of hatching, were released into one metre diameter field plots and allowed to complete development in the field. Groups of known numbers of similar ticks were put in tick-proof nylon mesh tubes (25cm x 2cm) and bags (5cm x 2cm). The tubes were placed upright in the vegetation in some of the plots. The bags were put under natural cover at soil level.

The plots containing the free ticks were divided into three groups, sampled weekly, monthly and once every four months. The last group was to exclude the possibility that the weekly and monthly sampling might remove all the ticks before they were dead. The ticks in tubes and bags were sampled every month and those found alive were applied to rabbits to feed in order to test their viability.

It is too early to draw any conclusions about adult survival, but conclusive results were obtained for larvae and nymphs released in phase I. There was considered to be a "peak" of tick activity (Table 3) if over 1% of the ticks estimated to be present in the plots was collected during flagging at any one time. The results shown in Figs 3 and 4 demonstrate survival patterns as measured by the numbers of active ticks of all stages sampled in phases I and II. The larvae and nymphs were very active soon after they hatched or moulted, but by the eighth week the number dropped considerably (Fig 3 and Table 3). Isolated larvae were still being picked up between weeks 18 — 27 when they finally disappeared. Although the number of nymphs picked up was initially high, they fell to very low numbers between weeks 20 — 39 and then disappeared. Adults also showed their highest activity soon after release but continued for at least 50 weeks. Activity did not increase in the rainy seasons.

For Phase II (Fig 4 and Table 3) the results were very similar to those in phase I. The larvae and nymphs showed a high level of activity soon after release. The larvae, however, were mostly dead by week 8, although occasional specimens were picked up until week 27. Very low numbers of nymphs were active up to week 27 and isolated specimen continued to be picked up until at least week 40. Adults were only collected in very low numbers throughout.

The results of the monthly sampling suggested similar patterns of survival to those indicated by weekly sampling. The larvae in phase I survived for a maximum of 5 months (Table 3) compared with 27 weeks for those under weekly sampling. Nine months survival corresponded well with 30 weeks survival for nymphs sampled once per month and

Table 3 Survival data for ticks introduced into enclosed plots on the ground in September 1979 and February 1980, and then recovered by sampling; a = accumulated percentage recovered, b = time interval to last recovery, c = times after release when there was peak activity. Results in brackets are incomplete as ticks still alive.

Tick instar	Weekly Sampling a %	b weeks	c weeks	Monthly Sampling a %	b months	c months	4-monthly Sampling a %	b months	c months
				Phase I (September 1979 — November, 1980)					
Larvae	69.1	27	1 — 13	37.3	5	1 — 3	2.3	4	4
Nymphs	67.2	39	1 — 22	35.4	9	1 — 6	7.2	8	4
Adults	(28.1)	(60)	3 — 6, 38, 39, 46	(8.3)	(14)	4, 10, 13	(0.2)	(12)	—
				Phase II (February 1980 — November 1980)					
Larvae	44.2	37	1 — 5	22.1	8	1 — 2	0.1	8	—
Nymphs	(38.1)	(40)	1 — 6	28.0	8	1 — 3	(1.0)	(8)	—
Adults	(13.3)	(40)	18	(4.8)	(9)	4	(0.8)	(8)	—

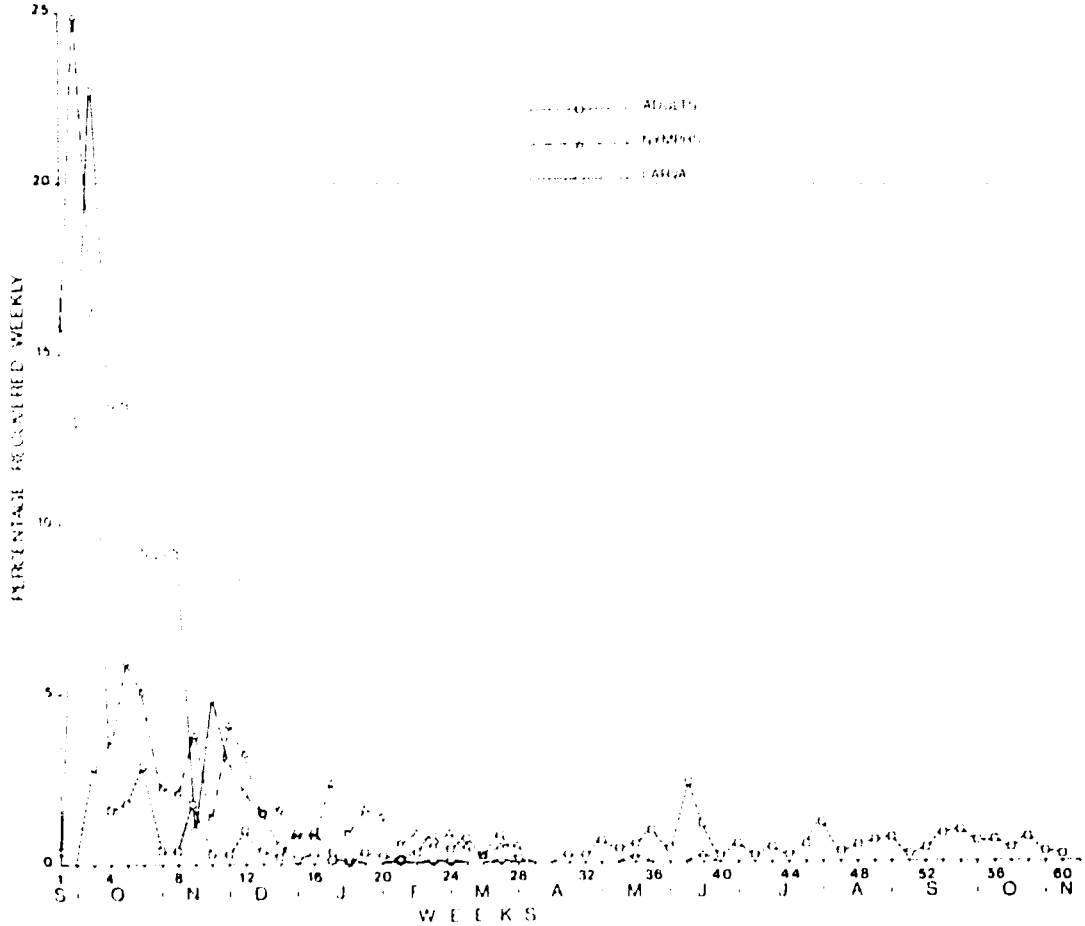


Fig 3. Survival of *R. appendiculatus* ticks in relation to their activity levels. Phase I

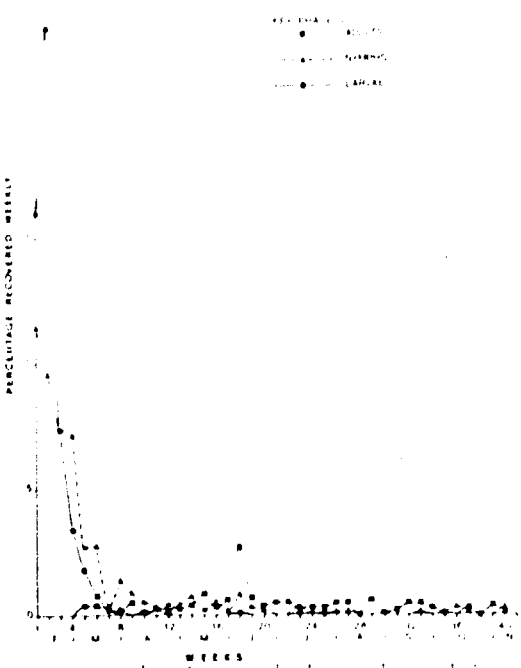


Fig 4. Survival of *R. appendiculatus* ticks in relation to their activity levels. Phase II

once per week respectively. In phase II, the maximum larval survival periods were eight months and 37 weeks.

The total numbers of larvae and nymphs collected in phase I were approximately one and a half times greater than in phase II, suggesting variations in the level of tick activity which may be seasonal.

The results from the plots sampled every 4 months (Table 3) confirmed that the disappearance of the ticks under weekly and monthly sampling was not caused by depletion of their numbers by sampling, but was due to death. For instance, the 2.3% of the larvae recovered during the first and only positive sample of phase I, confirmed that they were truely declining, as also indicated by the five month survival period seen in the monthly sampled plots.

The results from the ticks confined in nylon mesh tubes and bags were also very interesting. There was some agreement between the time of disappearance of the free ticks in the plots and the death of those in confinement. The surviving ticks always fed readily and completed their development, irrespective of their age.

In the more uniform conditions of high humi-

dity throughout the vegetation layers that occurred during the rainy seasons, the adults tended to congregate in the upper part of the nylon tubes, whereas they concentrated near the bottom part in the dry seasons. The larvae and nymphs did not show this seasonal variation, but always remained at the top until they died. This confirmed the observations in the plots with free nymphs and larvae that the numbers declined steadily after release without any appreciable later recovery. The dead ticks were mostly found at the bottom of the tubes.

The following conclusions can be drawn from the results.

1. Previous reports suggest that adult ticks, surviving in the field show peaks of activity (i.e. numbers collected) during the rainy seasons. In the present experiment the numbers of adults collected have so far been uniformly low, although during the rainy season the majority of ticks in the nylon tubes were found near the top.
2. Most natural populations show a peak of adult feeding activity in the long rains, giving rise to a relatively short period of high larval numbers, at a time when conditions on the ground are still favourable for their survival whilst waiting for a host on which to feed. The present results suggest that even if conditions are not favourable for survival the larvae and nymphs are unable to modify their behaviour and remain continuously active near the top of the vegetation until they quickly die.

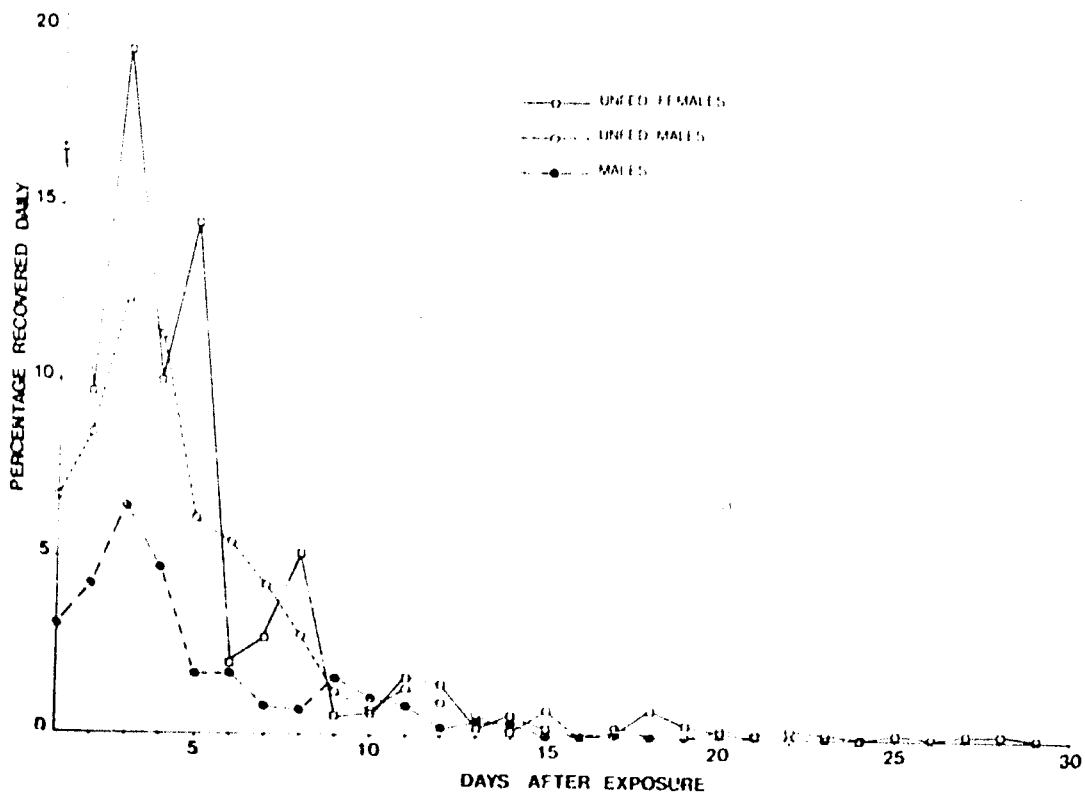
The feeding performance of ticks does not seem to decline with age. It is only the total population of ticks, still able to feed if the opportunity arises, that decrease.

Pick up rate of *Rhipicephalus appendiculatus* in the field

D.K. Punyua

It is important to know the rate at which ticks are picked up by their hosts under varying conditions in order to improve our understanding of tick population dynamics and also to be able to assess the effectiveness of methods of tick control.

Fig 5. Daily pick up rates of *R. appendiculatus* ticks in the field



Known numbers of fully hydrated adult ticks were released on the ground in two double fenced plots of 1000m^2 each. Twenty groups of ten ticks similar to these were put in nylon mesh bags (2cm x 5cm,) and placed in the vegetation at the edge of the paddocks in order to assess the likely survival pattern of the free ticks. One week later two bait cattle were introduced to graze in each plot. They were searched daily and all those ticks found were removed and counted.

Into one plot 1000 unfed females were released, and into the other 1000 unfed males plus 1500 males removed from the rabbit host on the seventh day of feeding. The latter were identifiable by the protruding caudal appendage. Ticks of this type can be recovered in samples collected from the vegetation.

The results are shown in Fig. 5. In all cases a majority of the ticks recovered was collected in the first weeks, and all the ticks so far obtained were taken within a period of two weeks (fed males), 3 weeks (unfed males) and 4 weeks (unfed females), with recovery rates of 26.0%, 69.5% and 56.8% respectively. For the ticks in the bags the corresponding mortalities over 15 weeks were 100%, 65% and 50%.

The cattle were withdrawn after seven weeks without picking up any more ticks. Thereafter a single animal was re-introduced for 24 hours once per week, checked for ticks and removed again. In this way resurgence of activity would be detected and the cattle would then be introduced continously to resume sampling. It is inferred that a proportion of the populations of unfed males and unfed females remained inactive on the ground, but that the fed males had all died.

Evidence for the role of juvenile hormone in the control of reproduction and the rate of moulting in *Ornithodoros p. porcinus.*

F.D. Obenchain, C.K.A. Mango and A. Bwire

Oogenesis, initiated after an *in vitro* bloodmeal of defibrinated pig blood is abortive in virgin argasid female ticks; eggs are absorbed before the completion of vitellogenesis and oviposition. In an investigation of the gonotropic potential of insect juvenile hormones we found that topical treatment with *cis trans, trans* isomers of JH-I or JH-III (10 µg/2µl acetone stereochemical purity about 85%) two days after feeding induced 5 of 18 and 9 of 28 virgins to oviposit, respectively; 19 solvent and 18 JH-II treated virgin females did not oviposit.

When female *O. p. porcinus* are fed *in vitro* on defibrinated blood with added ecdysterone (4µg/ml), even when mated, they usually begin to mature oocytes which are then adsorbed when the females supermoult. The adsorption process begins during a peak of moulting hormone activity (determined by Musca bioassay) which occurs 2 — 3 days before ecdysis. The following data demonstrate that exogenous insect JH-III can antagonize the promotion of supermoulting by exogenous ecdysterone fed females which had been mated prior to feeding, 12 supermoulted and 2 oviposited; 7 of 8 acetone controls (2µl) supermoulted and 1 oviposited. After topical application of lug of JH-III in acetone, 7 supermoulted and 0 oviposited, but 10µg doses of JH-III completely blocked supermoulting with 6 of 10 JH treated females ovipositing. These data support the hypothesis that argasid ticks use a JH-like compound as their gonadotropic hormone.

Fig 6 shows the effects of 10ug of JH-I (applied topically in lµl acetone) on the rate of moulting among sixth instar nymphal *O. p. porcinus.* The inhibiting effect is strongly dependant upon the time of application. When JH is applied at or prior to 48h after feeding, the 50% cumulative level of moulting is delayed by 2 — 3 days in comparison to untreated and acetone treated controls. When sixth stage nymphs were treated with JH-I (10µg/tick) 54h, 96h, 144h or 182h

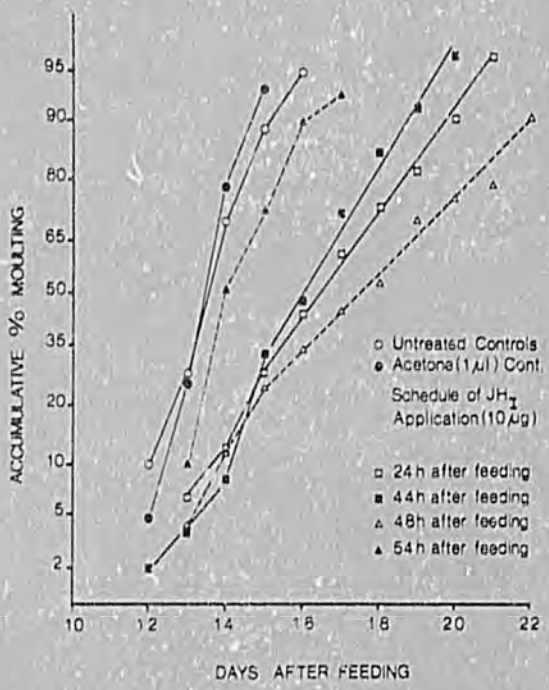


Fig 6: Gated sensitivity of *Ornithodoros porcinus* 6th stage nymphs to JH$_I$ induced moult delay

after feeding the rate of moulting was not substantially different from that observed among controls. These data suggest that the moult inhibiting effects of JH-I are strongly gated. By analogy to the apparent situation in insects, one role of a tick "juvenile hormone" may be to regulate ecdysteroid titres during the course of development. JH-I had no juvenilizing effects on treated sixth nymphs; there were no significant differences in the proportions of adult females and males or of seventh nymphs following any of the JH treatments by comparison with the controls.

These data strongly suggest that ticks use a compound similar to insect JH as their gonotropic hormone and may use the same hormone to regulate ecdysteroid-dependant developmental events.

Immunogenicity of larval extract from *Rhipicephalus appendiculatus* in rabbits

M.G. Binta

It has been reported that homogenates of whole ticks as well as of midgut and salivary gland used as antigens to immunize cattle and laboratory animals against other tick genera exerted deleterious effects on the feeding and reproduction of these ticks.

The purpose of this study was to investigate the potential of larval tick extract (see ICIPE Annual Report 1979) as a protective immunogen against ticks using the following adjuvants: 1) *Vaccarus stam*, 2) Complete Freund's adjuvant and 3) Alum. This may result in an immunization regimen incurring deleterious effects on both the feeding and the reproduction of various tick instars feeding on immunized rabbits.

Amblyomma aggregation attachment pheromones, hierarchy of behavioural responses

F.D. Obenchain and R. Opowa

Certain species of *Amblyomma* ticks, including several East African species (*Amblyomma variegatum*, *A. gemma*, *A. lepidum*, *A. cohaerens*, and *A. eburneum*) which parasitize cattle and may serve as vectors of various pathogens, show a complex pattern of interactions between feeding males and unattached females which regulate the attachment behaviour of the mated females. In the sequence of behavioural events: (1) mated males attach to preferred sites on appropriate hosts, feed for 7 to 10 days and mature sperm; (2) Unfed females do not attach to these hosts in the absence of fed males, but continue in an exploring type behaviour; (3) As males become sexually mature they also begin to respond to contact with exploring females by elevating their bodies to a perpendicular position on the host, extending their legs and displaying their ventral body surface; (4) Females of certain *Amblyomma* species (i.e. *A. variegatum*) show strongly positive chemotrophic responses from short distances and this leads to their aggregation in the vicinity of displaying males. Females of other *Amblyomma* species (i.e. *gemma*, *A. cohaerens*, *A. eburneum*) show positive, but less rapid aggregation responses to displaying males; (5) As opportunities arise, displaying males grasp exploring females with their outstretched legs and (6) rotate them, if necessary, so that the female mouthparts are directed toward the host; (7) When the females are held in this position, venter to venter with the male, they explore the gular region of the male with their palpal organs and then proceed to (8) extend their chelicerae, generate a bleb of milky salivary secretion, begin lateral cutting movements of the extended chelicerae digits, and penetrate the host epidermis.

This complex series of behavioural interactions may be coordinated by several discrete pheromonal mechanisms: (a) the fed male appears to release a short range pheromone which is sensed by receptors on the female tarsus I, leading to their aggregation in the vicinity of sexually mature males, and (b) second male produced pheromone may be associated with the cuticle or glands in the gular region which, after contact has been made with receptors of the female palpal organ, initiates her attachment behaviour.

In vitro and *in vivo* production of tick ecdysteroids

B. J. Ellis and E.N. Ole Sitayo

Ecdysteroid production both *in vivo* and *in vitro* by post-repletion nymphal *Amblyomma variegatum* was measured by radioimmune assay. A profile of ecdysteroid titres produced by individual organs explanted daily during nymphal development was compared to *in vivo* daily ecdysteroid titres of the whole ticks. Analysis of the culture medium supernatants revealed that only the fat body organ cultures had elevated titres and these increases appeared to be very similar in timing to that of the whole ticks *in vivo*. *In vivo* peaks occur at times of apolysis of the mouth and legs, and again at the time of dorsal apolysis.

Knowledge of the peak times of hormones produced and released as well as its source(s) will enhance the development of a system for *in vitro* culturing of the arthropod stages of the parasite that causes East Coast Fever. This information will also provide another target tissue, in addition to the salivary glands, to use in the control of ticks.

GRASSLAND TERMITES RESEARCH PROGRAMME

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Introduction

Termites are an integral part of the semi-arid grassland ecosystem where they function both as herbivores and decomposers. Their impact on the grasslands is two fold. They consume considerable quantities of plant material and move large amounts of soil. Except for periodic devastation by locusts, the major competitors of cattle and other vertebrate herbivores are the grass-feeding termites. Populations of some species approach the biomass of the large grazing animals and consume comparable amounts of forage.
showed that at least one species has the potential for becoming a pest in poor years since it may be forced by grazing pressure to switch from dead grass litter to standing grass or even to plant roots.

The Termite Programme is broadly committed to analysing the role of termites in a semi-arid grassland. It is unique in this respect since other termite research groups are concerned with taxonomy, basic biology, or else aimed at controlling or preventing termite attacks on wood and wood products. The ICIPE programme encompasses termite physiology, behaviour, and ecology including soil relationships, with long-term objectives being 1) to provide input for grassland management and, 2) to maintain a reservoir of data and ideas which can be used in developing environmentally acceptable control methods and innovative pest management practices.

In previous years physiological research has focused on the hormonal control of caste development, especially of soldiers and reproductives; behavioural studies have probed the role of pheromones in mediating trailing, defensive and trail following behaviour. Ecological work done at the Kajiado Field Station has dealt with the size and structure of populations, forage production, collection and consumption, termite predation by ants; and the physical-chemical properties of soils, soil fertility, and soil translocation by termites.

The role of *Macrotermes* in Soils

M.A. Arshad

Effect on soil productivity

Work initiated in 1978 to determine the role of termites (*M. michaelseni*) on soil productivity in a semi-arid grassland ecosystem, Kajiado, Kenya, was continued during 1980. This year's data confirmed the results obtained during 1978 and 1979. The major findings of this study are (i) the dry matter yield in the termite modified area at 1 – 10m distance from mound is about 2½ – 3 times greater than in the area beyond 20m which is relatively unaffected by the termites (ii) *Themeda triandra* which is the dominant grass species of the area is totally absent in the 1 – 10m zone around the mound. The major species in the immediate vicinity of the mound are *Pennisetum stramineum* and *Cynodon dactylon*, the latter become a dominant species (over 90%) within the 0 – 10m zone and is entirely absent beyond 15m from the mound (iii) The number of grass species increases from 2 – 3 species around the mound to 6 – 7 species in the area which is apparently beyond the influence of the mound (iv) High concentration of nutrients and favourable water availability together with good drainage were found to be the major causes of increased biomass and different species composition of vegetation around the mounds.

Mineral composition of plant species of the termite-modified area

Investigations into the chemical composition of various grass species common around termite mounds and in the adjacent area were carried out in order to assess their nutritional requirements and their nutritive values as forage grasses. Plant

Table 1. Mineral composition of plant species of the study area (Kajiado, Kenya)

Plant species	Ash		N		P		K		Ca		Mg		S	
	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf
	%													
Species dominant around termite mounds:														
Cynodon dactylon (L) Pers.	9.6	12.2	0.92	2.28	0.32	0.45	1.70	1.05	0.09	0.49	0.05	0.16	0.12	0.11
Pennisetum stramineum Peter	10.4	15.5	0.90	2.60	0.21	0.36	1.20	1.90	0.10	0.59	0.10	0.22	0.04	0.04
Species dominant in the region:														
Themeda triandra Forsk.	9.9	13.5	0.58	1.17	0.08	0.20	0.50	0.86	0.12	0.34	0.02	0.06	0.02	0.02
Digitaria scalarum Chiov	6.5	12.6	0.91	1.47	0.26	0.49	1.76	1.40	0.07	0.32	0.06	0.12	0.06	0.05
Other species common in the region:														
Eustachys paspaloides Lanza and Mattei	8.7	13.0	0.77	1.82	0.25	0.54	2.00	2.15	0.19	0.61	0.02	0.11	0.04	0.05
Chloris roxburgiana Trin.	12.1	14.7	1.13	1.94	0.19	0.43	2.35	1.60	0.17	0.52	0.05	0.10	0.07	0.06
Harpachne schimperi Hochst. ex. Rich	11.2	13.1	0.56	1.61	0.26	0.49	1.20	1.30	0.14	0.45	0.10	0.17	0.04	0.04
Aristida keniensis Henr.	10.5	15.1	0.98	2.10	0.17	0.22	1.10	1.00	0.12	0.42	0.06	0.11	0.06	0.06

samples were taken in early May, 1980, during maximum vegetative growth; this period normally coincides with the most intensive grazing by cattle. Stem and leaf samples were thoroughly washed with water, dried at 65°C and ground in a Wiley mill before chemical analysis. Results indicate (Table 1) that leaves of *Cynodon dactylon* and *Pennisetum stramineum* which are dominant grass species around termite mounds contain the maximum concentration of N (crude protein: 14.2% and 16.2% respectively) while leaves of *Themeda triandra* dominant species in the region have the lowest level of this element (crude protein: 7.3%). Although the levels of most major nutrients are higher in *C. dactylon* than in *T. triandra* the differences in levels of S content in the two species are very striking. Other species have an intermediate level of S. High S content of *C. dactylon* appears to be related to its physiological characteristics rather than S content of soil since no significant difference in concentration of this element between mound and adjacent soil (data not shown) was noted.

Effect on various soil types

Chemical and exchange characteristics as well as available nutrients of termite mounds developed on different parent materials in various eco-climatic regions of Kenya were studied. The results indicate differences in soil properties among mounds developed on the same parent material but in different eco-climatic zones. In general, termite mounds have much higher exchangeable calcium, cation exchange capacity, base saturation, iron and aluminium than the adjacent soils in all the eco-climatic zones. Available P is higher in the mounds located in the drier part of Kenya (Ecological Zones IV & V) than in the adjacent soil while the converse is true for the humid regions (Ecological Zone III). However, the majority of mounds and their adjacent soils investigated are deficient in available P. Mound soils invariably contain high amounts of NO3 — N particularly in the nursery and base of the mound. High temperature and moisture in the mound may be partly responsible for mineralization of organic N to NO3 — N as reported by Kozlova (1951) in Turkmenia. Continous washing and deposition of readily available N around the mound during rainstorms may result in better plant growth near the mounds (ICIPE Annual Report, 1979). Accumulation of soluble constituents in the termite mounds also indicates that leaching losses of available nutrients from the live mound are much lower than in a normal soil profile.

Further observations on external structures related to the nests of *Macrotermes michaelseni* in Kajiado

J.P.E.C. Darlington

Following the work on underground foraging passages and storage systems described in the last annual report, it was decided to investigate what happens when two nests are so close together that their foraging territories would be expected to overlap. The system selected consisted of two mounds 45 m apart, one a large mature mound and the other a rapidly growing young mound. Underground passages were traced from each nest towards the other. At a distance of 30 m from the old mound and 15 m from the young one, the passage networks from the two mounds joined up in four places through small (less than 2 cm. wide) passages, which did not appear to have been sealed in any way. Several storage pits in these

passages were full of the head capsules of dead *Macrotermes* major workers and soldiers, a feature never previously encountered in the underground passages.

The earlier studies had shown that an expanding *Macrotermes* colony will take over and incorporate the passages of nearby dead nests into its own foraging system. The present observations indicate that an expanding colony will trespass into the passage system of an active neighbouring colony, and that the latter may resist the incursion. This is the first direct evidence that territorial conflicts between neighbouring termite colonies take place underground. The young colony may be testing the strength of its neighbour, and if it experienced no resistance to its trespass, it would presumably proceed to take over part of the neighbour's system for its own use.

It was shown earlier that forage collected at night on the surface was stored temporarily in pits alongside the underground passages. A method has now been devised for sampling the forage directly from the pits on the morning after its collection. These samples can be used to determine the composition of the forage, but not to quantify the offtake, since an unknown amount of forage will already have been transported to the nest. Samples taken in October, 1980, before the start of the rains, consisted mainly of pieces of dry grass leaf and stems. Some of the samples also contained leaflets of *Acacia tortilis* which appeared to have been collected from the soil surface after having fallen from a nearby tree. The average dry weight of forage recovered from 50 pits was 0.22 g per pit. The volumes of cleared pits were measured by filling them up from a known volume of dry sand and measuring the sand left over. The mean volume varied between 2 and 5 ml per pit on different passages, with an overall mean of 3.7 ml per pit. Forage density was found to be 0.09 g dry weight per ml, so the estimated maximum storage capacity of a pit would be 0.33 g dry weight. This indicates that the pits sampled were filled fairly near to their maximum capacity.

The impact of feeding by *Macrotermes* on semi-arid pastures

N.M. Collins

The aim of this project is to determine the economic and ecological impact of *Macrotermes* on semi-arid pastures. Studies by M.G. Lepage showed that feeding by *Macrotermes michaelseni* may only be of economic importance during drought years. Under normal circumstances the species is a litter feeder and does not take green grass. Studies are being extended to include *Macrotermes subhyalinus* at a new study site near Bissel, 30 km south of Kajiado. Since the work began at the new site in June, 1980, insufficient data have accumulated to present valid quantitative conclusions. However, the site and constituent experiments may be briefly described.

The density, size and distribution of *M. subhyalinus* nests have been meadured on a 12 ha area. Mound size is correlated with nest populations and will be extrapolated to an estimate of the overall field population. The central 1 ha experimental site is divided into four plots, two fenced and two open. Biomass of standing grass and litter are measured monthly on each plot, to assess the grazing impact of cattle and game. Grass litter decomposition rates are estimated monthly, both within and without 2 mm mesh litter bags. Four plots of baits consisting of dung, wood and grass are examined weekly and show that *M. subhyalinus* is mainly feeding on grass, but is only one of a spectrum of species fully utilising the available foodstuffs. The quantitative impact of *M. subhyalinus* is monitored monthly by estimating the number of foraging holes opened per night and the amount of grass removed per hole opened. Foraging holes are monitored on 24 strips, each 1.5 x 10 m, in which holes are marked with cellulose filler and examined daily. Consumption is estimated using small (20 cm diameter) corrugated iron enclosures, in which known amounts of grass litter are left overnight around a single hole. If the hole is opened by the termites, then the amount removed is measured and compared with control enclosures.

The alates flew from the nests on the nights of November 15th and 17th, following the first heavy shower of the short rains on November 9th. Subsequently, the foragers began to feed in the open during the entire day, permitting detailed observations of their habits. The foraging parties consisted mainly of major workers and minor soldiers, with one or two major soldiers guarding each hole. Most of the grass forage is collected as litter on the soil surface, woody stalks being taken more frequently than leaves. Occasionally however, foragers may climb as high as 0.5m onto small woody shrubs or tussocks of the grass *Pennisetum*, which has a bushy growth-form. Although the termites may exceptionally cut a piece of green grass, they generally feed on the woody dead or dormant parts of the plant. Workers may be seen testing a green leaf by nibbling the edge of the lamina, but generally reject it at this stage. It is of interest that the rather woody *Pennisetum*, while apparently favoured by *Macrotermes*, is the last grass to be eaten by cattle.

Predation of Grassland Termites in the Semi-Arid Savannah at Kajiado by the Ant *Megaponera foetens*

G.H.N. Nyamasyo

The ponerine ant *Megaponera foetens* is an obligate predator of termites and it hunts in well organised raids. Its prey is composed mainly of the foraging workers and soldiers of termites of the sub family

Macrotermitinae. In order to estimate the impact of *M. foetens* predation on *Macrotermes* populations at Kajiado it was decided first to investigate in detail its nesting behaviour and also to study its population dynamics.

M. foetens — location and distribution of nests

The nests of *M. foetens* are simple excavations which open to the surface via two to four round holes. Some of the nests are found in old eroded termite mounds while others are made on ordinary ground. By measuring the distances between one *M. foetens* nest and its nearest neighbour — i.e. both *M. foetens* nests and *Macrotermes* mounds, it was found that the predator's nests are located in regular proximity to the termite mounds ($S^2 : \bar{X}=0.9$). *M. foetens* nests are positioned at a mean distance of 22.2 ± 4.4 m away from *Macrotermes* mounds. Such behaviour, where a predator lives within a fixed distance relative to its prey, not only implies obligatory predation but also suggests a certain amount of prey management by the predator. Thus the suitability of *M. foetens* nest site depends to a large extent on the position of a *Macrotermes* mound.

The population dynamics of *M. foetens*

Between 18 and 27 nests of *M. foetens* were monitored for a year from November, 1979, to October, 1980. The dead workers and pupal exuviae of this ant species were collected daily and counted. The workers were examined for any broken appendages. This method was developed after field observations had revealed that, during a raid by *M. foetens*, some of the ants incur appendages from *Macrotermes* soldiers. However, these victims are usually carried back to the nests and when they eventually die, are removed in the process of nest cleaning together with pupal exuviae and those dying from other causes, and scattered outside the nest entrances. This behaviour provides an easy means of studying the population dynamics of *M. foetens* directly in the field. Fig. 1 shows both the natality and the mortality of *M. foetens* as estimated from the collected exuviae and dead workers. The monthly natality or mortality is expressed as a percentage per nest of the total natality or mortality. Fig. 2 shows the total mortality s plit into conflict and non-conflict mortalities. Fig. 3 shows the rainfall pattern and also the temperature fluctuations throughout the study period.

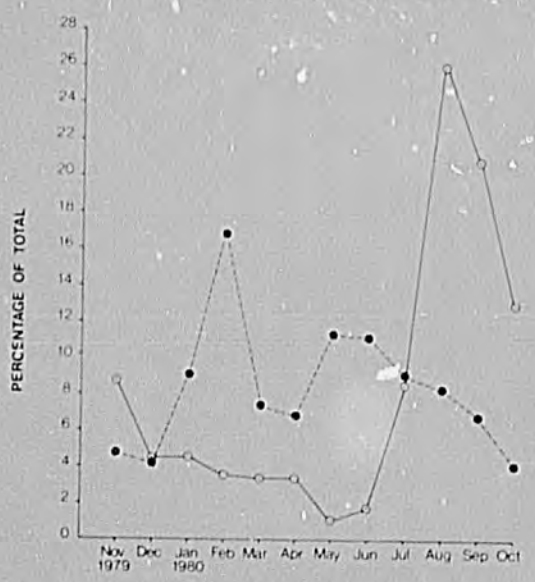

Fig 1: The natality and mortality curves for *M. foetens* O——O Natality ●---● Mortality (total)

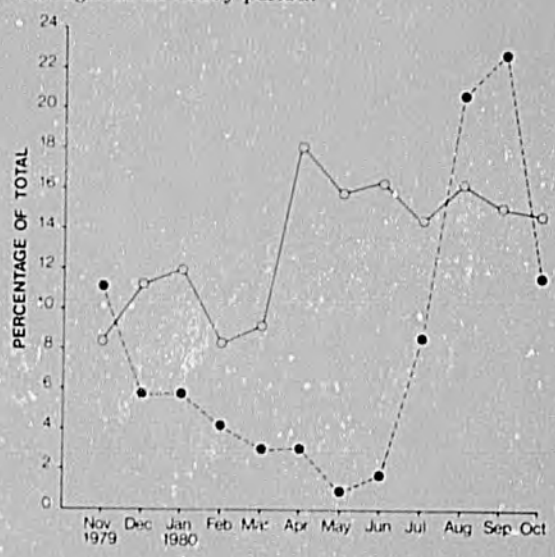

Fig 2: Mortality curves for *M. foetens* ●---● Conflict Mortality O——O Non-conflict Mortality

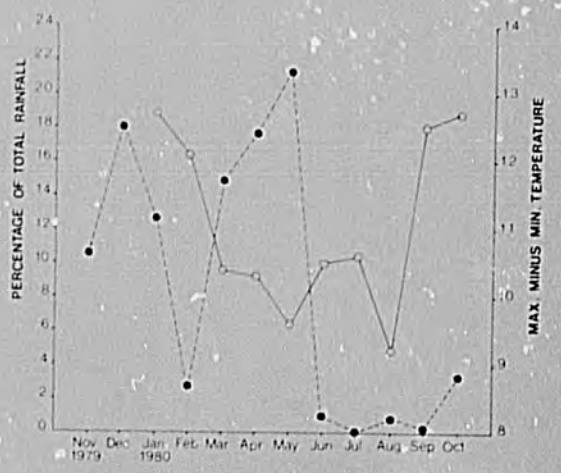

Fig 3: Rainfall fluctuations curve ●---● Max. minus Min. Temperature curve O——O

Although *Macrotermes* as food for *M. foetens* is not likely to be a limiting factor, particularly in view of the latter's nesting behaviour, the natality fluctuations can be explained in terms of prey availability. The influence of rain is complex. Probably small rains synchronise the foraging activities of both the prey and the predator (as all are likely to respond to the same environmental factors) resulting in a higher hunting success by the ants and hence their high natality. With too much rain the foraging activity of termites decreases or stops and this leads to a lower hunting success by the ants and thus the low natality. Nevertheless prey availability during the rainy season is higher than in the dry season. This is probably because of the availability of other termite species as prey during the rains. Complete absence of rainfall leads to an increase in foraging activity by *Macrotermes* (Lepage, ICIPE Annual Report, 1976). The decrease in natality during the dry season is possibly accounted for by the high temperature and high mortality of the ant workers, both factors resulting in less food available to the immature stages.

The total mortality is more or less constant during the rainy season but increases from May to a peak in August. The conflict mortality (i.e. dead ant workers with broken appendages) curve follows closely the total mortality curve. Thus the mortality fluctuations are usually a result of conflict encounters with *Macrotermes* soldiers. Non-conflict mortality is low during the rainy season but increases to a peak in April after which it remains more or less constant throughout the dry weather. It is speculated that the effect of dry weather on the population dynamics of *M. foetens* is, first, through the reduction of prey available to the ant larvae due to increased defence by the *Macrotermes* soldiers and, secondly, through increased dehydration of the ant workers, particularly those injured during a raid. The latter possibility would account for the high mortality during the dry season. It is nevertheless possible that weather fluctuations influence the population dynamics of this ant species in other ways. This study is intended to run for at least three years.

1. Trail specificity: Information content for recognition of own against alien trails among *Trinervitermes* colonies

G.W. Oloo

Specificity studies on pheromone systems are of biological interest mainly in field situations where different communities share the same habitat and are in a position to interact. It is also of interest to determine whether pheromones evolve along different lines over the geographical range of a species and whether similarities occur in different species. In the last Annual Report, it was shown that neighbouring colonies of *T. bettonianus* showed no preference for their own colony's trails in a choice situation. However, sympatric populations of different genera *Trinervitermes*, *Macrotermes* and *Odontotermes* were found to recognise their respective trails with a high degree of precision. Similar studies have been carried out with *Trinervitermes* spp. from different habitats.

Results and Discussion

In the competitive trail-following tests with well established natural trails and glandular extract trails, allopatric populations of *T. bettonianus* showed no significant preference for their own colony's trails in a choice situation (Table 2). In some cases, both colonies preferentially followed the same trail strength from colony to colony. Varying degrees of cross-following of trails was also observed between the closely related *T. bettonianus* and *T. germinatus*, *T. gratiosus* from East Africa, (Table 3) and *T. trinervius*, and *T. togoensis* from West Africa (Table 4).

These series of studies reveal cases of non-specificity of termite trail-following and a high degree of specificity between sympatric populations of different genera. Attempts are being made to analyse for possible increase in colony-specific trail information from colony population to species level in *Trinervitermes*.

Table 2. Selective trail-following: Studies with well established natural trails (M_1, N_1) and glandular extract trails (M_2, N_2) of allopatric populations of *T. bettanianus* from Machakos (M) and Narok (N).

Source of trail & test termite	Trail-following response + Replicates (colony pairs tested)						
	I	II	III	IV	V	VI	VII
M_1	16*	9	15*	13	12	2(**)	8
N_1	7	4	6	11	12	19**	12
M_2	11	17**	4(**)	2(**)			
N_2	12	7	19**	17**			

+, +ve choices of own colony's trail (out of 20 choices) against alien trail. * *, Significant preference at $P<0.05$; **, $P<0.01$; (**), preference for alien trail.

Table 3 Interspecific trail following – Analysis with well established natural trails of *T. bettonianus* (Tb) from Machakos and *T. grativelus* from Kibwezi

Source of trail and test termite	Trail following response + Replicates (colony pairs tested)						
	I	II	III	IV	V	VI	VII
Tb	16*	19**	12	19**	16*	16	20**
Tg	10	12	20**	6	7	19**	6

+, *, ** (see Table 2 for explanation)

Table 4 Interspecific trail following – Analysis with glandular extract trails of *T. bettonianus* (T. bett.) from Machakos, *T. grativelus* (T. grat.) from Kibwezi, Kenya, and *T. geminatus* (T. gem.), *T. trinervius* (T. trin.) and *T. togoensis* (T. togo.) from Nigeria.

Source of trail & test termite	Trail following response + Replicates (colony pairs tested)				
	I	II	III	IV	V
T. bett T. grat	14 8	16* 7	19** 4(*)	14 16	12 16*
T. bett T. gem	14 9	12 12			
T. bett T. trin	15* 12	19** 14			
T. bett T. togo	12 10	10 9	15* 12		
T. grat T. gem	9 15*	7 13			
T. grat T. trin	8 14	10 8			
T. grat T. togo	17** 13	16* 11			

+, *, (*) (see Table 2 for explanation)

II. Polymorphism of the sternal gland of the worker caste of *T. bettonianus*

As far as is known, postembryonic development and activity of the sternal gland in relation to its function in various castes or age groups within a colony have been studied to a limited extent in one or two termite species only. In the specificity studies reported above marked differences in trail-laying capacity from colony to colony were sometimes observed. It was further noted from field observations that the proportion of different age groups of workers participating in the foraging activity varies from one colony to another. The work reported here aimed to determine whether there are any differences in the size and activity of the sternal gland in various (7) instars of the worker caste of *T. bettonianus*.

Results and Discussion

Significant differences in size and potential trail-laying activity of the sternal gland were observed in different instars of the sternal gland of the worker caste of *T. bettonianus*. Instars 1 and 2; 2 and 3 showed no significant difference in size; there was a significant difference ($P<0.05$) between instars 1, 2, and 3 as a group; 4, 5, 6 and 7 (Table 5). Thus the glands of the 7 instars fall into 5 distinct groups of size. The sternal gland showed no detectable activity in the 1st instar; gland activity increased 1000-fold from 1st – 7th instar. (Table 5). Field observations indicated that instars 5, 6 and 7 constitute over 90% of foragers (sampled from 12 colonies); hence, their relatively high gland activity may be related to their trail-laying behaviour during foraging. The observed variation in trail strength from colony to colony may be partly influenced by the proportion of the different age groups participating in foraging.

Table 5 The Sternal Gland: Variation in size and activity in different instars of the worker caste of *[illegible]*

Instar	No. Individuals observed	Mean Gland size ($\times 10^2$ (μm^2))	No. colonies sampled	Mean Gland Activity (Trail Units)*	Range (Trail Units)*
*LW$_1$	18	9.1	8	0	
LW$_2$	17	12.6	8	4.0	3 – 5
LW$_3$	27	16.5	8	18.3	10 – 20
LW$_4$	20	23.7	8	150.0	100 – 200
LW$_5$	22	35.9	8	300.0	
LW$_6$	12	45.3	8	437.5	400 – 500
LW$_7$	30	53.9	12	716.7	600 – 1000

* LW$_1$ – LW$_7$ = 1st – 7th instar of large (female) worker

* One trail unit is defined as the minimum amount of glandular extract trail necessary to elicit significant ($P<0.05$) t-test following response when applied at the rate of 5µl/10 cm on paper substrate

Rank (Gland Size)

LW$_1$ = 1, 2, 3, 4, 5, 6, 7

Preliminary observations on growth related events in the epidermis of third instar *Macrotermes michaelseni* (ISOPTERA) in relation to changes in endocrine glands

R.M. Oloo & Sieber

Results have now accumulated on developmental pathways and the role played by the endocrine system in caste differentiation. We have recently shown conclusively that juvenile hormones are important in soldier determination and that differentiation under the influence of the hormones may occur only in the third instar during a definite interval within the intermoult. In the present study, work was therefore initiated to elucidate the mechanisms of hormonal action in the differentiation. We started with histological investigation of a possible relationship between epidermal events and changes in activity that occur in the endocrine glands during an intermoult period of third instar female larvae.

Third instar larvae of known ages were processed histologically and both corpora allata (CA) and prothoracic glands (PG) were measured. Histological changes occurring in the epidermis of the same individuals were also noted. Changes were found in the sizes of both CA and PG during the intermoult period (between 0 and 13 days). There are two distinct peaks of activity (CA and PG size). The first ones occur on day 2 of the instar when epidermal detachment from old cuticle on the mouthparts starts.

On the third and fourth days, the glands decrease in size in the same pattern now with all larvae having mouthparts with detached cuticle. A rapid increase in size of CA follows while that of PG is slow. Corpora allata attain the largest size on the sixth day corresponding with initiation of general detachments of cuticle in all parts of the larvae. Two days later, the PG also reach maximum size which coincides with the beginning of epidermal cell proliferation and deposition of new cuticle. A day or so later, heavy deposition of new cuticle follows. These enlarged CA and PG start to decrease and continue so throughout the remaining period of the instar. Moulting is initiated on about the 12th – 13th day soon after which wax deposition occurs.

Another aspect of work, initiated in collaboration with Chemistry and Biochemistry Research Unit (Dr. P.G. McDowell), concerns the identification of juvenile hormones in immature stages of *Macrotermes*. This work, coupled with juvenile hormone (JH) treatment of third instar larvae, will give us some insight into the differential effect (Specificity) of JH on caste determination, the study that will follow. A more detailed study will be conducted to confirm the preliminary results reported above with additional work on hormonal assays of larvae through the intermoult period.

Queen replacement and development in *Macrotermes michaelseni*

R. Sieber

Queen replacement

This experiment was designed to find out whether a field colony can survive after the death of the reproductives, i.e. whether a colony without reproductives would produce another royal couple. To take into consideration the varying composition of colonies at different times of the year, the royal cell was removed from 24 nests in November when the colonies contained alates, and then from 8 nests every second month throughout one year. After six months these mounds were reopened and observed whether the royal couple had been replaced and whether nymphs had developed at an unusual time of the year.

Results showed that unusual nymph production never occurred and that the development of a new royal couple is possible but exceptional. In only 5 out of 64 cases was a new royal cell found, containing two queens and one king. Although these reproductives had been accepted as the new royal pair for six months only, the two queens showed distinct signs of physogastry: the body weight was about half that of a mature physogastric queen, JH-titre and corpora allata (CA) had clearly increased. Also the number of egg producing ovarioles was comparable to that of a physogastric queen. These findings show that the size of the colony plays an important role in inducing the physogastry of queens.

Whether the physogastric queen was replaced immediately or 1, 2 or 3 weeks later by young laboratory reproductives, the newly introduced reproductives were in no case accepted. Replacement of reproductives in field colonies was sometimes successful, when royal cells, containing the physogastric queen and the king were exchanged between field colonies. This is contradictory to the case with incipient colonies, where reproductives can easily be exchanged.

The physogastry of queens

The physiological changes of the reproductives in incipient colonies were investigated to find out why physogastry occurs in females in adult field colonies but never in incipient colonies in the laboratory. As mentioned above, the size of the colony is apparently an inducing factor. Thus far this seems to be confirmed by preliminary studies of young reproductives during a period of 200 days after flight: the number of offspring reaches a certain level while JH-titre, volume of the CA and number of active ovarioles remain at a more or less constant level.

Water intake by *Macrotermes michaelseni*

R. Sieber

Behavioural observations have shown that the hypopharynx is always protruded by termite reproductives and workers during water intake. To determine the function of the hypopharynx, its fine structure was investigated by means of SEM and thin sections. The surface of the hypopharynx which is pressed onto the wet surface while drinking is covered by a dense mat of hairlike structures. The thin sections show that these structures are trichomes. It is therefore assumed that water is taken into the mouth cavity, not by active transport but, by capillary force.

The path of the water was followed by morphological investigations in major workers. Water is firstly stored in the foregut. It is then transferred via the haemolymph to the water sacs which have been found in all the other castes of *M. michaelseni*. The duration of water intake in workers deprived of water for 15 h was measured. No relationship between the duration and the amount of water imbibed could be found. In contrast, the ratio of imbibed water to body weight of thirsty workers is almost constant. This indicates that the hypopharynx is protruded until a definite amount of water is imbibed.

TSETSE RESEARCH PROGRAMME

Programme Leader

Dr. A. Challier (1978)

A. Tsetse Reproductive Physiology Project

Dr. M.F.B. Chaudhury (1974) Senior Research Scientist
Dr. M.S. Ramasamy (1979) Research Scientist
Mrs. R.W. Kunyiha (1976) Research Assistant
Mr. F. Mukunza (1973) Junior Technician
Mr. P.A. Osula (1978) Junior Technician

B. Tsetse Ecology and Epidemiology Project

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Mr. R. Mutuaruhiu (1979) Junior Technician
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C. Trypanosome-Vector Physiology

Dr. I.H. Otieno (1973) Senior Research Scientist
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Miss R. Chesang (1972) Junior Technician
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General Introduction

Research is conducted to acquire basic knowledge in the fields of biology, ecology, and vectorial capacity of tsetse flies with a view to improve existing control procedures and promote possible new and environmentally safe methods.

The Tsetse Fly Programme consists of three projects:

- Reproductive physiology, aimed at better understanding of reproduction mechanisms that play an important role in the rate of increase of population.
- Ecology and Epidemiology, a project dealing with population dynamics and behaviour of flies both of which are important in the project design and implementation of tsetse control programmes.
- Trypanosome–Vector Physiology, aimed at elucidating the mode of action of factors involved in the vectorial capacity of flies. Field studies are carried out on *Glossina pallidipes* Austen, an important vector of both human and animal trypanosomiasis in East Africa. Most observations and experiments in the laboratory involve *G. morsitans morsitans* Westwood, an easily bred tsetse species.

Ecology and Epidemiology

Introduction

The main objective of the ecology programme is to develop a predictive population model useful in the understanding of tsetse fly population dynamics in various ecological situations. Such a model, devised for *Glossina pallidipes* Austen might later on be used for other species of *Glossina*.

The studies on population dynamics, including fluctuations and structure of population (sex and age), reproductive and nutritional status, and dispersal, are being conducted on the South Kenya Coast and in the Lambwe Valley, two study areas showing different habitat types and fauna.

Behaviour of flies, including resting and breeding sites, activity and host preference is also being investigated in relation to biotic and abiotic factors.

Population sampling techniques is another component of the programme aimed at developing a methodology by which catches of flies can be related to true population size and structure, and at finding a means to detect ultra-low population densities. In the first instance, experiments are being carried out to investigate the effect of revolving screens, with a view to improving the performance of the biconical trap.

In addition, trypanosome infection rate is currently being assessed in relation to changes in tsetse populations in five different epidemiological situations on the South Kenya Coast. Data collected will be useful for an eventual epidemiological model.

The Ecology of *Glossina pallidipes* Aust. in the Lambwe Valley, South Nyanza

D.A. Turner

Studies started in 1979 on the ecology and behaviour of *Glossina pallidipes* in the Lambwe Valley are continuing. Changes have been taking place in the Lambwe Valley in recent years which appear to have resulted in a seriously deteriorating epidemiological situation with respect to both livestock

and human trypanosomiasis during 1980. Chief among these are (1) extending tsetse habitat, characterised by progressive replacement of tsetse-free grassland by Acacia woodland in the floor of the valley, and colonisation by tsetse of a coniferous plantation, started in 1965, on the eastern valley wall; (2) an increase in the wild animal population of the Game Reserve in the centre of the valley as a result of anti-poaching measures; and (3) considerable increase in human settlement around the periphery of the Reserve.

Population dynamics

Monthly sampling of flies, using biconical traps, is being carried out to study the dynamics of populations in relation to climate, vegetation and food supply. The theme of this main, long term work is ultimately to derive models of populations under natural and under control conditions. For comparative purposes three localities are under investigation: a classical habitat of dense thicket in the middle of the valley; an atypical habitat afforded by coniferous plantation; and Acacia woodland, situated intermediately. In thicket and woodland, inside the Game Reserve, there is an abundant game population. The coniferous plantation is partly inside, partly outside the Reserve, being bisected by a boundary fence. The sampling area is located on the outside. Here hosts are much fewer in number. The main parameters used to detect population change are relative density, assessed in terms of catch per trap per day, and adult mortality as a function of the age structure of the (female) population, determined by ovarian dissection.

On the basis of little more than 12 months' observations it is too early yet to detect trends in population change. Some complications have arisen, moreover, due to the occurrence of chance factors operating during the sampling interval. These result in ephemeral fluctuations in the number of flies caught, which obscure underlying trends in population change. Such chance factors include sudden changes in weather (rain) grass fires, and transient movements of host animals.

While the majority of trap-caught flies have the appearance of being hungry (exact nutritional status needs to be determined by fat and haematin analysis), about 0.5% are actually engorged on capture, or show signs of having a substantial quantity of undigested blood in the gut. From these, blood meal samples are collected for serological indentification to determine host preferences and availability. Identifications from 400 blood meals have been made so far, and the results show that almost half derive from buffalo and bushbuck (26% and 21% respectively), probably more, taking into account the relatively high percentage (18%) which could only be classified as unidentified bovid. Other bovid sources include duiker (10%), roan (2%) and reedbuck (3%). 13% of feeds were taken from suids (bushpig and warthog), and the remainder (7%) from man plus unidentified primates, probably man. The latter figure is quite high for *G. pallidipes*. There were no discernable differences in feeding patterns with habitat type, except that bushbuck feeds were more common in thicket than woodland, which presumably reflects the habitat preference of this species. Available hosts not apparently fed on include impala, oribi, waterbuck and hartebeest.

In addition to population studies by routine monthly sampling, mark/release/recapture experiments have been carried out in a relatively isolated section of conifer plantation, to estimate, principally, population size. The data also yielded information on population structure and fly behaviour, which further enabled deductions to be made concerning biases in trap catch compostion. Similar estimates of population size were obtained from calculations derived basically from Lincoln Index methods. In a catchment area of approximately 30 hectares, the mean population sizes of non-teneral males and females were 10,467 (± 5710) and 15,297 (± 3481) respectively. The estimated total fly density, including tenerals, was 1,000 per hectare. Variability about the mean estimates was largely on account of changes in the availability of flies for capture, which reflected changes in their hunger cycle. Changes in recapture rate following mark/release suggested a three day feeding interval for both sexes. The sex and teneral/non-teneral composition of trap captures were in similar proportions to those obtaining in the natural population determined on the basis of estimated populations, suggesting that biconical traps suffer no serious sampling bias. From ovarian dissection, the estimated mean age of females was 49 days and the maximum age 255 days, and from the average number of pupae which could be produced it was deduced that the population was in a steady state. The mean age of males, deduced from wing fray, was 23 days. The greater longevity of females explained the difference in male and female population sizes derived from population estimates. Data obtained at the same time on pregnancy state showed that captures were deficient in females carrying a third stage larva. Otherwise, the biconical trap gave good representative catches in terms of female age structure. An abortion rate of 2.8% was detected, indicating that this contributes little to overall mortality in the population.

A repeat experiment was carried out in the same area one year later, using a more sophisticated approach to population estimation (Jolly's method). The results of this remain to be analysed.

Other sampling methods

Following a report on preliminary observations on the successful use of water traps for sampling

tsetse in Nigeria, a trial was carried out in the Lambwe Valley to determine whether these can be used to sample *G. pallidipes*. Catch comparisons were made over a four day period between eight differently coloured water traps, set up in two groups of four, and a single biconical trap placed, on alternate days, in the centre of each group. The water traps altogether caught only 12 flies compared to 784 by the biconical trap. The water trap is clearly ineffective in sampling *G. pallidipes*.

Experiments were also carried out to investigate possible olfactory attraction of low molecular weight compounds to *G. pallidipes*. Using a graduated flask and wick arrangement hung inside biconical traps, the effects of ammonia, acetone and acetic acid were tested in a 5 x 5 Latin Square experimental arrangement. No significant improvement in catch resulted from any of the chemicals tested. Similarly for a repeat experiment using ammonia and an acetone/ammonia mixture evaporating at a five times greater rate (40ml./h).

Screens impregnated with synthetic pyrethroids (decamethrin) have been found to be an effective, inexpensive and environmentally safe means of reducing tsetse density around foci of human trypanosomiasis in West Africa. Such a method may achieve a degree of control of *G. pallidipes* around settlements in the Lambwe Valley presently experiencing sleeping sickness. Preliminary trials have been carried out to assess the relative attractiveness of different coloured screens, coated with sticky substance (Tanglefoot) in this instance. Blue screens were found to be considerably more attractive than black, white, red or yellow.

Tsetse ecology on the Kenya Coast

W.F. Snow

The study of aspects of the population biology, including population dynamics, reproductive strategies and general ecology, of *Glossina pallidipes* on the south Kenya coast has continued.

Monthly samples, using biconical traps have been taken at Muhaka, Shimba Hills National Reserve and Ukunda Veterinary Research Station although the latter was cleared of tsetse habitat around May 1980 and observations were discontinued. Occasional visits have also been made to a farm at Diani and Mwalewa Forest near the Tanzania border. Fly density and population fluctuations are evaluated, in relative terms, from a geometric mean catch per trap per day. Reproductive age and insemination and abortion rates are assessed from examination of the ovaries of female flies from trap samples. Many aspects of the work are a continuation and consolidation of observations noted in previous ICIPE Annual Reports.

By the end of 1980 continuous monthly observations on population fluctuations and age structure will have been completed for 30 months at Muhaka, 20 at Shimba Hills and more limited periods at Ukunda and Diani. These data remain to be analysed in detail but some preliminary inferences can already be drawn.

Fluctuations in numbers of *G. pallidipes* are not synchronised between the study localities although they are no more than 20 km apart and climatic differences between them must be marginal. The apparent density of the tsetse population varies greatly from locality to locality with mean monthly catches of 1.2 and 51.7 females from Ukunda and Shimba Hills respectively at the two extremes of density. However, the mean survival rate determined from the reproductive age of samples of female flies is not significantly different between sites. These observations indicate that the factors which are regulating these *G. pallidipes* populations are operating independently and in a different way in each of the study areas.

From a preliminary analysis of the population monitoring and age-grading data it is clear that the rate of population change is related to the survival of the adult component of the population. Tsetse live longer when the population is increasing and die earlier when it is on the decline. Data from Muhaka is presented in Figure 1 which shows the correlation between population change and survival.

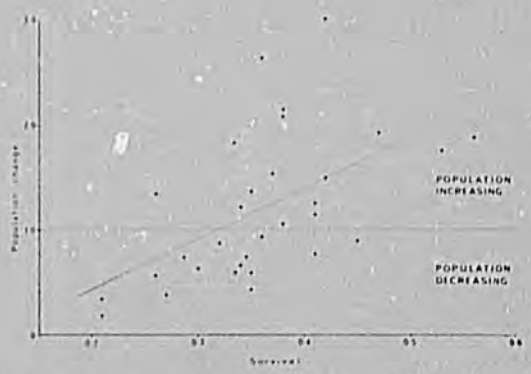

Fig 1: Population change (I) of *G. pallidipes* females related to relative survival(s) indicated by the age composition of samples from Muhaka.

$$I = \frac{\text{catch month}_n}{\text{catch month}_{n-1}}$$

$$S = \frac{\text{No. flies in reproductive classes } 5+6+7}{\text{No. flies in reproductive classes } 1+2+3+5+6+7}$$

Whilst it appears that population fluctuations can be largely explained on the basis of adult mortality patterns, it must be borne in mind that mortality at the pupal stage, which has not yet been investigated, and of newly emerged, teneral flies, which are not sampled efficiently by biconical traps, also contributes to the overall regulation of a tsetse population.

The factors which are involved in the regulation of numbers of tsetse in the study areas are being investigated in detail. These include both abiotic and biotic factors. As noted in the previous report, and supported by a further year's observations, rainfall of between 70 and 200 mm during the previous month is generally associated with an increase in numbers of *G. pallidipes* at Muhaka. The population declines with more or with less precipitation. A similar relationship cannot, however, be demonstrated for the data from Shimba Hills. It is certain that humidity, best expressed as saturation deficit, is a major determinant in tsetse ecology and rainfall is related to this in a general way. However, as commented above, the study areas are sufficiently close to suggest that climatic factors are not the cause of differences between the sub-populations of *G. pallidipes*. There is a general relationship between *G. pallidipes* numbers and the wildlife population of an area. Shimba Hills and Mwalewa harbour abundant wild hosts in large areas of semi-natural habitat with very large *G. pallidipes* populations. At Muhaka, Ukunda and Diani tsetse densities are much lower in association with small forest relics and with domestic livestock and small wild-pig populations as hosts. It is therefore apparent that biotic factors including habitat characteristics and area, host availability and direct density-dependent mortality through predation and parasitism are the cause of differences in the size and patterns of fluctuations between the sub-populations of *G. pallidipes* on the south Kenya coast.

Among females before their first ovulation, 2 — 4 and 6 — 8 day-old groups predominate in biconical trap catches. As reported last year, the former are almost exclusively virgin and the majority of the latter are inseminated. This indicates that female *G. pallidipes* only become sexually receptive or attractive to males after their first blood-meal. Understanding of the role of the contact sex pheromone of *G. pallidipes*, being investigated by the ICIPE Chemistry and Biochemistry Research Unit, may be the key to understanding this aspect of their behaviour. Pooled data from all localities give an ovarall insemination rate of 80% by day 8—9, although a few flies apparently ovulate without insemination (Figure 2). This may be the result of matings with old, impotent males. In contrast, almost 90% of *G. austeni* are mated by day 4 (Figure 2). Although there is evidence of considerable sexual activity by *G. austeni* in the cages of biconical traps and around 15% of females in the 0a age category contained spermatophores, only three out of 1273 *G. pallidipes* taken before their first ovulation contained spermatophores. Confinement in the small cages of biconical traps appears to inhibit sexual activity by *G. pallidipes*, or the situation lacks a stimulus essential for triggering mating behaviour. When samples of *G. pallidipes* are ranked by apparent density, it is clear that insemination rates are correlated with population density and are significantly lower in low density populations (Figure 3). This is undoubtedly related to the frequency of male/female encounters.

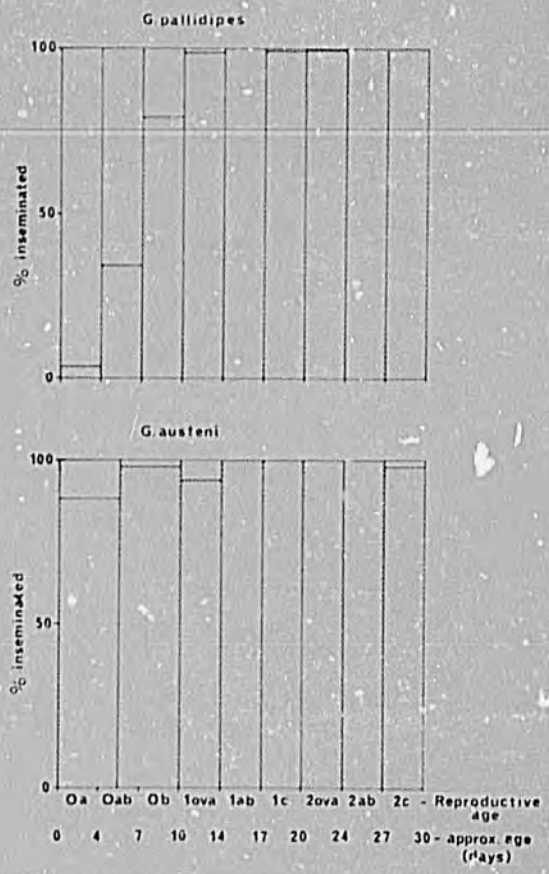

Fig 2: Insemination rates in female *G. pallidipes* (pooled data from all south Kenya Coast study sites) and *G. austeni* (from Muhaka) related to reproductive age.

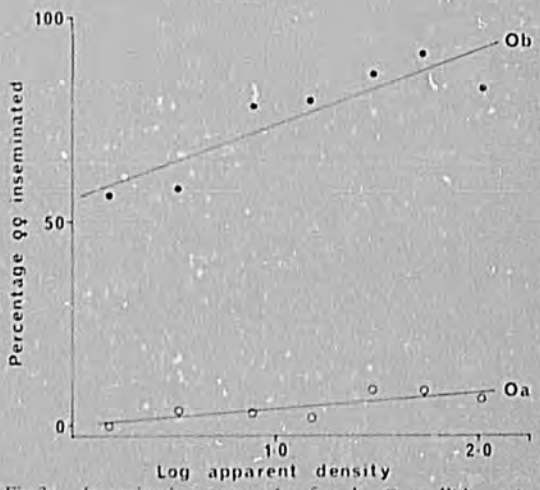

Fig 3: Insemination rates in female *G. pallidipes* in relation to apparent density. Densities indicated by catch/trap/day have been grouped with 7 arbitrary classes : 0–2, 2–5, 5–10, 10–20, 20–40, 40–80, 80+.

High abortion rates (up to 19%) are observed during the hot, dry seasons when large catches of tsetse are taken in the biconical traps. This is almost certainly an artefact due to confinement in the trap cage and a special sampling programme will be necessary to evaluate natural abortion rates. Nevertheless, it seem that abortions fall into two categories. In the commoner situation it may be better termed premature larviposition, although these larvae are immature and invariably succumb to environmental stress. The second less common situation, where flies abort much earlier, may be related to normal emaciation at the time of the first ovulation, sperm depletion or loss of sperm viability. It may be noted that the two flies in category 1c indicated in Figure 2 a not have been inseminated but also aborted.

General observations on the distribution and ecology of tsetse in relation to the epidemiology of trypanosomiasis on the south Kenya coast have confirmed four species of tsetse occur in the south coast area of Kenya. *G. pallidipes* is the commonest and most widespread. It appears to be highly adaptable and is present in both primary and secondary (bushed fallow) *G. austeni* and *G. morsitans* are only common in areas of relatively much tilled bush land and are retreating as a result of habitat destruction to create more farmland. In the mid-Tsavo basin of the mainland *G. longipennis* occurs, with *G. pallidipes* and *G. austeni* in moister areas. Sleeping sickness is absent, but challenge to livestock from nagana varies from zero in some intensively developed areas near Mombasa, from which tsetse have been displaced, to very high levels where large populations of *G. pallidipes* persist.

Sampling Techniques for *Glossina pallidipes*

M.E.A. Owaga and A. Challier

Past sampling studies with the biconical trap showed that trap catches were deficient in teneral and engorged tsetse flies. A slow moving vehicle gave higher yields and more teneral.

An experiment was designed to introduce movement into the biconical trap. A revolving component consisting of two screens, a dark blue and white one, mounted on a common straight holder was attached to the trap (Fig. 4). The holder was connected to a coil and the unit driven by a 12volt battery. The trap pole passed through a hole in the holder. While the latter revolved, the former (trap) remained stationary, so that the trap itself did not revolve. The speed of the revolutions was controlled as desired. A timer was used to interrupt revolutions and produce intervals during which the screens were stationary. Two series of experiments were conducted each consisting of six observations. In the first series a set of three traps was used, one with screens revolving at 40 Rev/min, another at 20 Rev/min and a third with stationary screens. In the second series of observations the speeds were altered, and the three traps had 35 Rev/min, 20 Rev/min with one minute interval and 0 Rev/min respectively.

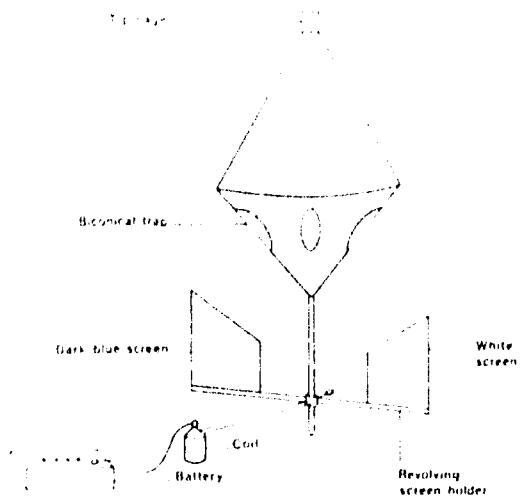

Fig 4: Biconical trap with revolving screens

Direct observations were carried out on each trap and records were kept of tsetse flies that landed on the trap those that departed, as well as those that eventually entered the trap. The flies caught were age graded, and the males were hunger staged.

The total yields and sex ratio of the tsetse are presented in Table 1. During the first series of observations the 0 Rev/min trap consistently gave best yields, both in total numbers and with regard to sex ratio. All the traps caught more males than females, but the 0 Rev/min trap had the highest number of females.

The 20 Rev/min trap caught the most tenerals, none trapped engorged flies.

During the second series of observations, the reduction in speed from 40 Rev/min to 35 Rev/min, and the one minute interval in the 20 Rev/min trap resulted in improvement in the yields by these two traps, and the catches from the 20 Rev/min trap were early but not quite as high as those from the 0 Rev/min trap. The 0 Rev/min and the 35 Rev/min traps got 5 and 6 flies respectively in hunger stage 3, that is some half digested blood in the gut. This constituted 0.5% and 0.8% respectively, of the flies caught by these two traps. No engorged flies were captured by the 20 rev/min trap.

All traps caught mostly females bearing egg/larva in developmental stages 'a' and 'b' (37 – 43%). The 35 Rev/min trap had the highest number of females (16%) bearing larva in stage

Table 1: Total Yields and Sex Ratios of *Glossina pallidipes*

	TRAP	NON TENERALS		TENERALS	
		♀♀	♂♂	♀♀	♂♂
FIRST SERIES	0 REV/MIN	326	484	27	16
	20 REV/MIN	207	252	22	40
	40 REV/MIN	154	220	20	38
SECOND SERIES	0 REV/MIN	562	445	8	14
	20 REV/MIN WITH INTERVAL	511	477	12	26
	35 REV/MIN	355	365	16	26

Table 2: Direct Observations on *G. pallidipes* Landing on, Leaving and Entering Traps

	TRAPS	ARRIVALS	DEPARTURES	ENTERED	ENT. STR.	HEAD DOWN
FIRST SERIES	0 REV/MIN	145	87	36(23%)	6%	8%
	20 REV/MIN	74	54	8(11%)	25%	4%
	40 REV/MIN	64	40	7(11%)	100%	2%
SECOND SERIES	0 REV/MIN	257	142	50(20%)	12%	4%
	20 REV/MIN WITH INTERVAL	197	132	36(17%)	19%	5%
	35 REV/MIN	103	36	28(27%)	46%	1%

ENT. STR. = Entered straight

'e' of development, that is with fully developed breathing lobes. Proportion of females with empty uterus was highest in the 20 Rev/min trap (15%), and lowest in the 0 Rev/min (5%).

Table 2 presents the results of direct observations. About 20 to 25% of all the *G. pallidipes* that visited the 0 Rev/min trap entered it, in the other two traps much less. A high proportion of the tsetse flies that entered the 40 and 35 Rev/min trap did so without landing on the cone 'entering straight'. 80% of flies that entered the 0 Rev/min trap first landed on the blue cone, loitered around and then eventually went in.

The age structure of females is presented in Fig. 5. The distribution from the 0 Rev/min trap is similar to that usually prevailing from the biconical trap without screen attachments, in that there is a peak in the middle age group, (categories 2, 3, and 4 instead of '0' and '1'). The 40 Rev/min had a similar peak and a deficiency in groups '0' and '1'.

However, when the speed was reduced to 35 Rev/min during the second series of observations the '0' category shot up, providing two peaks. The 20 Rev/min with interval, gave the best slope whereas group '0' had the largest number followed by group 1 and 2 etc. It would seem that teneral and male flies come to traps at an optimum revolution speed provided that the fly-flap effect of screens is suspended for a short time. Observations on these experiments are continuing.

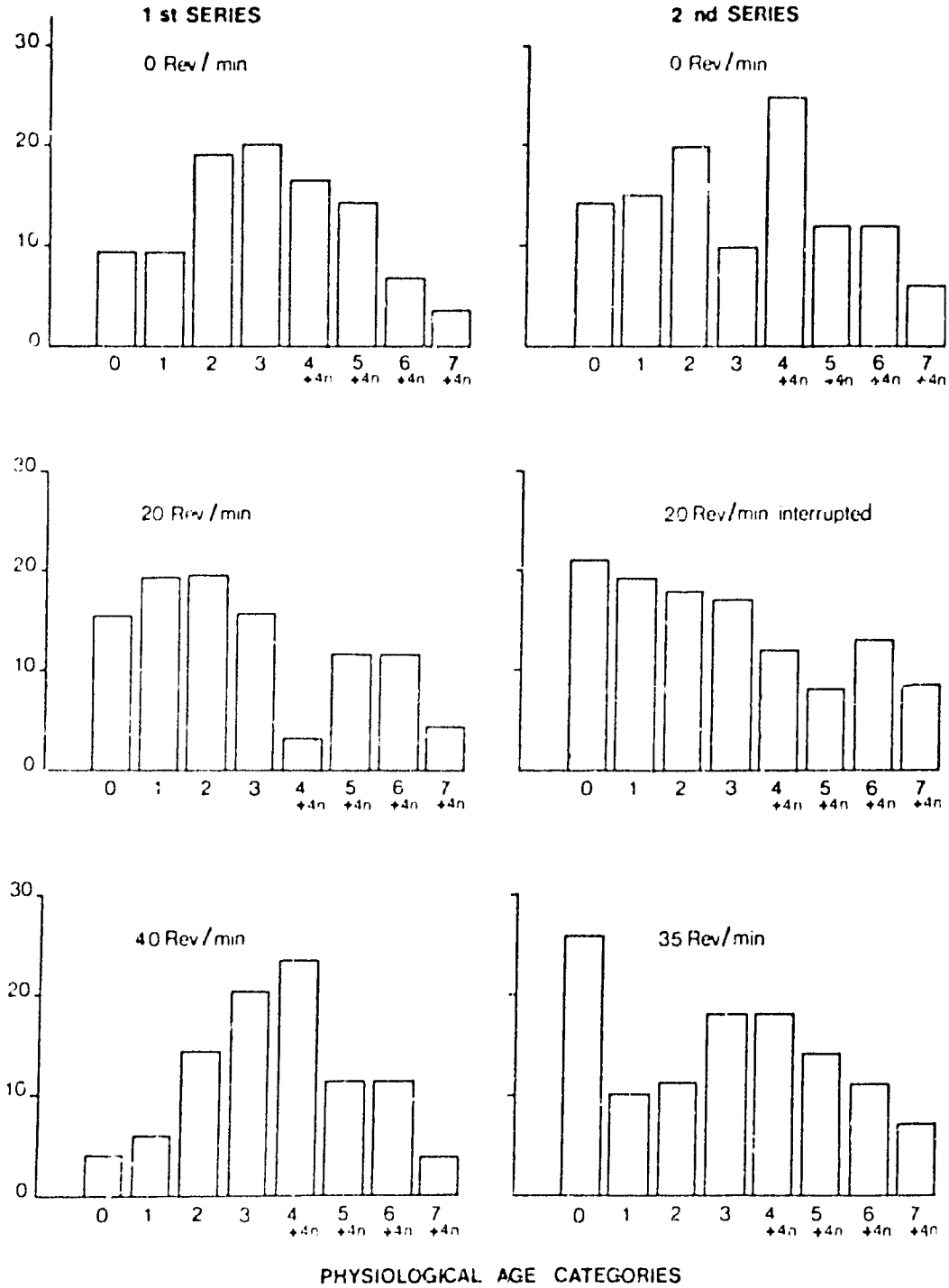

Fig 5 — Age structure of female *G. pallidipes* from traps.

Cuticular Lesions in Tsetse Flies of Kenya

D.A. Turner, W. Otieno M. Odindo, G.P. Kaaya and E. Kokwaro

Melanotic lesions, which appear to the naked eye as black spots, were first noted on the ventral abdominal integument of *G. pallidipes* from the Lambwe Valley. Surveys have subsequently shown these to be present in all tsetse species in Kenya (*G. pallidipes*, *G. swynnertoni*, *G. austeni*, *G. fuscipes*, *G. brevipalpis* and *G. longipennis*), and in all populations of these species sampled so far, from areas widely apart. No previous mention of such lesion is found in the literature. The prevalence of lesions ranged widely from 3 to 72%—

and varied with species, sex, locality and season. In all species lesions were more frequent in females than males.

Considerable variation exists in form, number and size of lesions. The commonest form is a pit or crater in the surface of the integument (Fig. 6), numbering between one and fifty or more on a fly, and in size from 0.02mm to 0.2mm. Another form is a raised scab of hard, black substance, undoubtedly melanin (Fig. 7), usually found singly and fairly massive in size. A variation of this is a large tumour-like eruption of soft cuticle with a deep core of melanin (Fig. 8). Other forms include large plates raised above the integument (Fig. 9), and, in marked contrast to the above, long, narrow tracks or striations of necrotic tissue, usually running anterior posteriorly along the integument (Fig. 10). Occasionally flies were seen with most of their abdomen encrusted with lesions (Fig. 11). All forms may be present on the same fly, and no differences were noted in form and number of lesions between tsetse species.

Fig. 6. — [illegible]

Fig. 7. — Melanin scab

Fig. 8. — Tumour with core of melanin

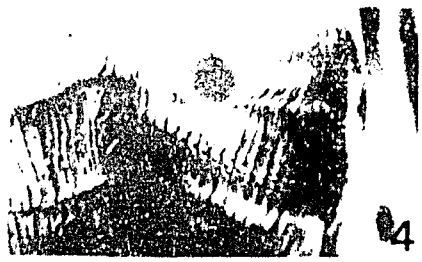

Fig. 9. — Plate form

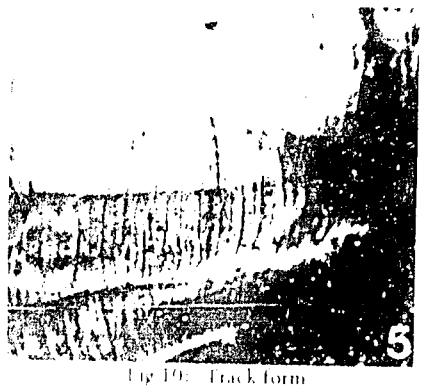

Fig. 10. — Track form

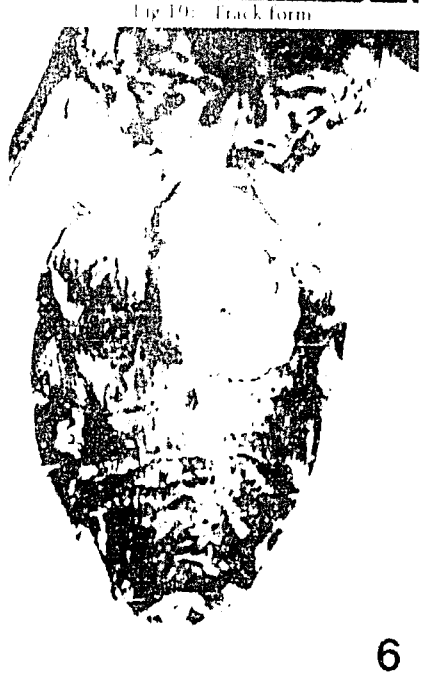

Fig. 11. — Abdomen half encrusted with lesions

Figs. 6 — 11. — Lesions on the ventral abdominal integument of *Glossina palpalis*

To determine whether a relationship exists between the presence of lesions and possible pathogenicity, a comparison was made between the population age structure of female flies of *G. pallidipes* exhibiting lesions and those without, using the ovarian age grading method. Lesions were found mainly in older flies (age category 4 and above), and none in very young flies (nulliparas), which indicated that lesions were not associated with any early mortality. When dissecting females, no evidence of lesion formation or of melanotic encapsulation was observed in internal organs (reproductive system, gut, fat bodies), nor any presence of parasitic macrostructures. Haemocyte studies showed that the white lesions had twice the haemocyte count of those without lesions. No differences were found, however, in the proportion of haemocyte types.

Attempts made to further isolate micro-organisms which could be responsible for lesion formation have been unsuccessful. Lesions dissected out and introduced into planted nutrient media which support the growth of a wide variety of bacterial and fungal pathogens induced no fungal or bacterial growth. Further microbiological and histopathological tests are being carried out to investigate other possible etiological agents. There is also the possibility of lesion formation and subsequent melanization resulting from scouring of the integument by non-infectious agents. Two instances have been observed of large foreign bodies piercing the integument and remaining *in situ* at the time the fly was captured. Massive melanization had taken place around the wound.

Patterns of Trypanosome Infection in *Glossina pallidipes* on the Kenya Coast

S.A. Farano

There is no information on infection rate in *Glossina pallidipes* in the Tsetse Ecology project on the coast. This study was therefore initiated in February 1980. Five localities were chosen on the basis of habitat and patterns of host availability.

Diani and Ukunda, semi-rural areas few wild animals.

Shimba Hills and Mwalewa, natural habitat with wild hosts.

Muhaka, an intermediate situation with both domestic and wild animals.

The infection rate and proportion of trypanosome species present were observed quarterly; during the dry season (February), rainy season (May), intermediate rains (August) and during the short rains (November). Factors affecting infection rate such as age of fly, climate and host availability were considered. The reproductive age of female flies was determined by examination of their ovaries. The data obtained so far suggest:

- A relationship exists between the age of a sample and infection rates. If the population is living longer it will take more blood meals and consequently has a higher infection rate resulting in higher challenge incidences.
- Areas with wild animals appear to have a lower infection rate than domesticated areas. It is possible that wild animals have lived with the disease for many years and thus have evolved a mechanism for suppressing it.
- *G. pallidipes* on the coast feed on both bovids and suids. Even in rural areas wild pigs form an important blood source. The parasites observed so far are, in order of abundance, *Trypanosoma congolense*, *T. vivax* and *T. brucei* (Table 3). These parasites have been identified through their location in the fly. With the exception of Diani, *T. vivax* appears to be more prevalent in areas with wild animals while

Table 3. Percentage trypanosome infection rates in *G. pallidipes* from localities on the southern Kenya Coast.

	Rural areas		Wild areas		Intermediate
Trypanosome species	Ukunda	Diani	Shimba Hills	Mwalewa	Muhaka
T. congolense/T. simiae	11.7	3.4	3.4	5.7	8.0
T. vivax	0.3	2.2	3.3	0.9	1.1
T. brucei	0.3	0.0	0.4	0.0	0.0
TOTAL %	12.3	5.6	6.8	6.6	9.1
No. of flies examined	412	1066	1187	1063	1233

T. congolense in the dome treated areas. *T. brucei* is rare, and has been found in only two localities: Shimba Hills and Ukunda. *T. simiae* has in the past been identified on the coast. Since *T. simiae* and *T. congolense* occupy the same location in the fly, methods are being sought to separate these two related species.

The results from Diani (Table 3) appear odd and further investigation are underway to find out why the infection rate is very low compared to Ukunda only 2 km away. It is possible that regular chemoprophylaxis given to the cattle at Diani could explain the trypanosome infection rates observed in *G. pallidipes*.

REPRODUCTIVE PHYSIOLOGY

Introduction

Tsetse flies reproduce by adenotrophic viviparity, giving birth, at regular intervals, to single fully developed larva. Cyclical events of reproduction such as egg maturation, ovulation, larval development in the uterus, secretory activity of the uterine glands which supply nourishment to the developing larva, and larviposition are regulated with marked accuracy by the female tsetse. The combination of the low rate of fecundity and the complexities of the reproductive process can be considered a vulnerable feature of the tsetse physiology which, when fully understood, may be exploited in formulating novel control strategies. The objective of the research was to study various events of tsetse reproductive process in order to understand the underlying mechanisms which regulate the complex events of the pregnancy cycle. The research projects undertaken during the course of the year are presented below.

Control of Milk Gland Activity in Tsetse

M.F.B. Chaudhury, F. Osula and R.W. Kunyiha

The milk gland of *Glossina* sp. consists of a ramifying series of tubules in the abdominal cavity. This is a highly modified accessory gland and the cells of this show morphological features typical of secretory cells. Previous findings clearly demonstrated that removal of the corpus allatum results in gland tubule shrinkage whereas, replacement therapy with juvenile hormone III (JH III) or juvenile hormone mimic (JHM) reversed the situation (ICIPE Annual Report, 1977) suggesting that the uterine gland activity is regulated by JH. Present studies were undertaken to determine the effect of continuous hormone replacement therapy of allatectomized females and observed the effect of the application of JH on the milk gland of virgin females.

Teneral females of *G. morsitans morsitans* were allatectomized 6 hrs. after emergence and were allowed to mate after 2 blood meals when they were 2 days old. Each female was treated topically with 2 µg of JH III in 1 µl of acetone on day 3 following emergence and every alternate day thereafter until the end of the second cycle. Virgin females were treated with 5 µg of JH III in 1 ul of acetone per female twice a week for 4.5 weeks (period for 2 normal reproductive cycles). Average diameter was obtained form ten samples of distal tubules of the milk gland from samples of each age group.

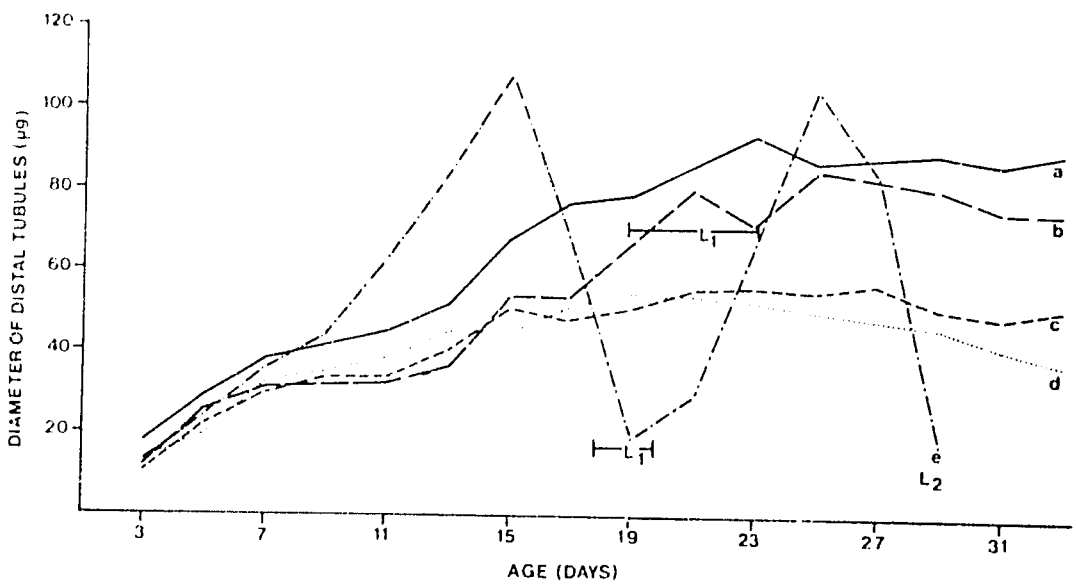

Fig 12: Diameter of distal tubules of the milk gland of females of indicated age which received various treatments, a, JH III treated virgins; b, allatectomized and JH III treated mated females; c, untreated virgins; d, acetone treated virgins, e, mated, untreated normal females; L1, time of first larviposition, L2 time of second larviposition

Results (Fig. 12) show that the distal tubules of the milk gland of the allatectomized and mated females treated with JH III did not show cyclic activity typically shown by those of the normal untreated pregnant females; instead, the tubules in most cases remained relatively large following the first larviposition. Additionally, examination of some of the tubules revealed copious secretions. However, it was observed that the tubules showed typical cyclic activity if the application of JH was discontinued before the end of the cycle.

Distal tubules of the treated virgin females slightly increased in diameter during the first and second week of the treatment. However, the diameter of the tubule was found to be significantly higher than those of the untreated control or acetone treated flies during the third and fourth week. Presence of milk was apparent in many of the tubules examined. These preliminary observations suggest that the JH is probably involved in the induction of milk synthesis although the exact role of the hormone is unknown. Additional factor(s) may also be involved in regulating gland activity since a maximum diameter of distal tubules normally observed in normal pregnant flies was never detected in either allatectomized hormone treated or virgin hormone-treated flies.

Further research at the histological, histochemical and ultrastructural level are in progress to understand the role of JH in the activity of the milk gland and the synthesis of milk. Possible role of brain neuro-secretory system in the control of milk synthesis is also being investigated.

Reproductive Abnormalities in Juvenile Hormone Mimic Treated Tsetse

M.F.B. Chaudhury, P. Osula and F. Mukunza

A female tsetse produces only one offspring at every 9–10 day interval as a full grown larva. The larva develops within the uterus of the fly by obtaining nourishment from the milk gland. Previous studies demonstrated that allatectomy results in the reduction of the milk gland diameter which in turn results in the production of non-viable or diminutive offspring. It has also been shown that the normal pregnancy cycle of the tsetse can be disrupted by using insect hormones and their mimics (ICIPE Annual Report 1979). The objective of the present study was to determine the effect of ZR 515, a juvenile hormone mimic compound, on pregnancy and the milk gland activity in the tsetse fly *G. morsitans morsitans* with particular reference to the time and dosage of application.

Each female was treated with 5 µg of the compound in 1 µg of acetone per treatment on day 1 (24 hrs. after first larviposition), 4 and 9 of the second reproductive cycle. Multiple treatments were administered on above days in various combinations.

Results summarized in Table 4, show that ZR 515 was most effective in inducing abortion of larvae and eggs when applied on day 1 as well as day 4. Most of the abortion occurred within 48 hrs. of the application. Many aborted eggs did not show any sign of embryogenesis. Examination of the gut contents and weighing of the aborted individuals did not reveal any state of malnutrition and the milk glands of the treated females examined were found normal. Applied on the 9th day of the pregnancy cycle, the mimic did not have any effect on the current pregnancy cycle; however, such application arrested embryogenesis in eggs of some treated females and prevented eggs with fully grown embryos from hatching in other such females.

The above results indicate that only one application at the beginning of the cycle is not effective in inducing abortion; instead, an application at the time of hatching or multiple applications during embryonic development appears to be more effective. Observation on the milk glands of the treated females resulting in abortion suggests that application of ZR 515 did not adversely affect the actitivity of the milk gland. The fact that the

Table 4. Reproductive abnormalities in *G. morsitans morsitans* resulting from topical application of ZR 515

No. of Treatment[4] (Day of Pregnancy Cycle)	N	% Females Aborted		% Females with Eggs	
		Egg	Larva	Showing Embryonic Arrest	Failed to Hatch
1 (Day 1)	50	18.0	14.0	16.0	0
1 (Day 4)	24	0.0	29.0	0	0
1 (Day 9)	40	*10.0	5.0	*35.0	*15.0
2 (Day 1 & 4)	28	17.9 *14.3	46.4	0	0
3 (Day 1, 4 & 9)	46	13.0 *17.4	71.7 *8.7	*23.9	*15.2

[4] All acetone treated controls (1 µl/treatment) produced normal larvae.

* Occurred in the third cycle

milk glands of the treated females examined were normal, indicates that the cause of abortion could not have been inavailability of nutrient resulting in larval starvation. It is possible that the hormone mimic inhibitance induces premature release of a larviposition factor (possibly a neurohormone), which in turn induces abortion. Phenomena such as arrest of embryogenesis and prevention of egg from hatching are perhaps two manifestations of morphogenetic effects of a juvenile hormone mimic although very little is known regarding the role of JH in embryonic development of insects.

Sexual Maturation in Male Tsetse

M.S. Ramasamy

Preliminary studies on control of reproduction in male *Glossina morsitans morsitans* were reported in the ICIPE Annual Report of 1979. Subsequently further studies were carried out to elucidate the role of endocrine factors and their possible interaction with other physiological processes during sexual maturation. Experiments with pharmacological agents were designed to obtain information on the role of biogenic amines during copulation and insemination.

The differences between accessory reproductive gland (ARG) secretions of teneral and 15 day old males is quantitative and the inability of males to successfully inseminate during early days of adult life can be attributed (among other factors) primarily to the insufficiency of ARG secretions. The number of blood meals has a direct influence on the quantity of ARG secretions, which in turn, determines the amount of sperm transferred during copulation. Sodium dodecyl sulphate polyacrylamide gel electrophoresis shows that all six major proteins of ARG secretions are present in the spermatophore and no major proteins are detected.

The synthetic activity of the A cells of the paraintercerebralis of the brain was measured by ratio of nuclear/cellular diameters. Increases from emergence until day 6 and then declines. An almost perfect second degree curve can be fitted to these points.

The rate of increase of nuclear diameter exceeds that of cell diameter up to day 6, after which the rate of increase of cell diameter exceeds that of nuclear diameter. It is likely that a build up of the appropriate concentration of medial neurosecretion in circulation is necessary for the manifestation of sexual behaviour and that by day 6, hormone in circulation has built up to an effective concentration, when conditions for copulation and insemination are optimal. Injection of 'brain extracts' made from sexually mature males increases the inseminating ability of young males; these extracts do not, however, induce synthesis of ARG secretions.

Experiments involving surgery have confirmed that the ability to synthesize/incorporate ARG secretions and their subsequent transfer to the female and also sperm transfer are independent of the presence of the corpus allatum or its hormone. Neither is an intrinsic secretion of the corpus cardiacum involved in these processes. Topical application of precocene I or precocene II, does not affect the synthesis/incorporation of ARG secretions and the copulating and inseminating abilities of the adult male.

The axon endings on the ARG contain dense core granules which appear to be aminergic. The plant alkaloid, reserpine, affects monoaminergic transmission, by causing a long lasting depletion of amine stores in the peripheral and central nervous systems.

Reserpine treated male will copulate, but they are unable to transfer ARG secretions or sperm. However, reserpine treatment on day 1 of adult life does not prevent the synthesis of ARG secretions. Due to the nature of reserpine action, it has not been possible to reverse its effect with monoamines (dopamine, 5-hydroxytryptamine or octopamine). Studies with other drugs interfering with monoaminergic/catecholaminergic transmission are in progress; preliminary studies indicate that catecholamines may be necessary for transfer of ARG secretions and also for sperm transfer.

Effects of Precocenes on Tsetse Reproduction

M.S. Ramasamy and M.F.B. Chaudhury

Precocene I and precocene II, isolated from *Ageratum houstonianum* are known to affect corpus allatum activity and consequently oocyte development in several insects. It was therefore of interest to study the action of these compounds on the reproductive processes of an insect reproducing by adenotrophic viviparity.

Females were treated with either precocene I or precocene II by topical application, before and after mating, and also after each larviposition. Precocenes do not affect mating and the size of blood meal in pregnant females. Neither is the incidence of larviposition/abortion significantly different from acetone treated controls. The duration of the inter-larval period and the pupal weights of pupae produced by precocene treated females are comparable to those of controls. Histological studies on the corpus allatum of precocene treated females do not show any degeneration of cells.

In the F1 generation, all females that emerge from pupae produced in the 1st reproductive cycle of precocene treated females have an oocyte in the right ovary. Retardation of oocyte maturation is observed in some of these females on day 9 of adult life. All F1 generation females which emerge from pupae produced in the 2nd and 3rd reproductive cycle of precocene treated females are normal in all respects. These flies copulate and are inseminated, but have no oocyte in either the right or the left ovary.

They contain only the germarium and the follicle does not descend even on day 9. Some female offspring which emerge from 4th cycle pupae of precocene treated females have no oocyte in either the right or left ovary while others carry a fully chorionated egg in the right ovary on day 9.

In tsetse, precocene I and precocene II do not affect the reproducing capacity of treated females. However, the effect of precocene manifests itself in some females of the F1 generation and this study constitutes the first report on the ability of precocenes to induce sterility in the F1 generation following treatment of the female parent. The 'sterilising' effect of precocenes is specific to female offspring, while all males of the F1 generation and males and females of the F2 generation produced by mating F1 males with untreated females are able to mate normally. In the laboratory each male inseminates over six females.

Topical application of juvenile hormone restores complete oocyte maturation in F1 generation females of the 1st cycle and reduces the incidence of sterility in 2nd, 3rd, and 4th cycles. Further studies on factor(s) causing sterility in females of the F1 generation are in progress.

Newly deposited larvae were treated with prococene by the contact method. Both female and male flies emerging from these pupae are normal and fertile.

Trypanosome Vector Physiology Project

The role of ambient temperature on infective development of *T. brucei* in *Glossina morsitans*

L.H. Otieno

Preliminary account of the response of *G. morsitans morsitans* to *T. brucei* infection was given last year (see 1979 ICIPE Annual Report). It was noted in that report that the ambient temperature at which freshly fed young tsetse flies were kept influenced the number of flies eventually developing mature salivary gland infection. An extension of this work is outlined in this report.

It was important to establish whether the good response of flies to trypanosome infection after a brief period of chilling following an infective blood meal was due to temperature *per se* or to the slow rate of crop emptying as has been suggested by Jenni (1977).

Fourteen C57B1/6 male mice were inoculated intravenously with stabilate TRUM 89 *T. b. brucei* 10^4 trypanosome/ml and on days 4, 5, 6 and 8 post inoculation, four uniformly infected mice were fed upon by young *G. morsitans*. Two groups of flies, 22–24 and 48–50 hour old flies exposed were divided into three groups, a, b, c. Soon after engorgement group b was transferred to a refrigerator maintained at 5°C for 1hr 30min, group a and c were kept at 25°C and 80% relative humidity.

After cooling (ostensibly to delay the rate of crop emptying) group b was returned to 25°C, 80% r.h. insectary. Six hours after engorgement group c was transferred to the refrigerator (5°C) and similarly cooled for 1 hr. 30 min. after which the three groups were kept at 25°C and 80% r.h. and maintained on two rabbits throughout the experimental period. The flies were dissected between 35 and 42 days post infected blood meal and examined for the presence of trypanosome infection. The results of these studies are shown in Table 5. The most significant part of these studies was the observation that 22 – 24 hour old flies cooled six hours after engorgement had the largest number (8 our of 26) with salivary gland infections. This was in sharp contrast with flies cooled soon after feeding or control non cooled flies which had 2(8.3%) and 1 (4.6%) flies with mature infections respectively.

The experiment was repeated using TRUM 273, a first passage derivative of TRUM 89 *T. brucei*. Young flies were fed each occasion on two rats showing rising parasitaemia after which the rats were killed and trypanosomes collected, used to feed through silicone membrane, another

Table 5: Effect of cooling freshly fed young *G. m. morsitans* on the eventual development of *T. brucei* salivary gland infections

Age of flies (Hrs) at infected feed	Control flies (group a)	Flies cooled soon after infected feed (group b)	Flies cooled 6 hrs. after infected feed (group c)	Total infections
22 – 24	1/22	2/24	8/26	11/72
48 – 50	1/19	1/25	0/24	2/68
Total	2/41	3/49	8/50	13/140

Numerator denotes number of flies with salivary gland infection.
Denominator denotes number of flies examined.

Table 6 Counts of trypanosomes resuspended in defibrinated rat blood, and an estimation of the numbers of stumpy forms present during the rising parasitaemias is shown

Infection in Rats	Trypanosome counts, ml of blood	% stumpy forms
Day 3	5.8×10^6	15.3
Day 4	4.2×10^6	22.4
Day 5	5.5×10^6	49.7

Table 7 A comparative study of *T. brucei* infection rates in *G. morsitans morsitans* following exposure to *in vivo* and *in vitro* feeding.

Infection in Rats (Days)	Control flies		Flies cooled soon after infected (10 hrs) feed		Flies cooled six hours after infected feed (6 hrs.)	
	In vivo feeding	*In vitro* feeding	*In vivo* feeding	*In vitro* feeding	*In vivo* feeding	*In vitro* feeding
3	$\frac{5}{18}$* (27.8)	$\frac{0}{15}$	$\frac{2}{17}$ (11.7)	$\frac{1}{9}$ (11.1)	$\frac{5}{25}$ (20)	$\frac{3}{14}$ (21.4)
4	$\frac{8}{31}$ (25.8)	$\frac{0}{10}$	$\frac{6}{27}$ (22.2)	$\frac{5}{16}$ (31.3)	$\frac{6}{27}$ (22.2)	$\frac{5}{14}$ (35.7)
5	$\frac{1}{23}$ (4.4)	$\frac{4}{16}$ (25)	$\frac{9}{25}$ (36)	$\frac{7}{15}$ (13.8)	$\frac{1}{21}$ (4.7)	*
TOTAL	$\frac{14}{72}$ (19.4)	$\frac{4}{44}$ (9.1)	$\frac{17}{69}$ (24.6)	$\frac{13}{41}$ (31.7)	$\frac{12}{73}$ (16.4)	$\frac{8}{28}$ (28.5)

* Flies died due to some technical error

* Fractions refer to number of flies with mature infections over the number of flies examined.

Figures in parentheses denote percentage of flies showing mature salivary gland infections

set of young flies. Both *in vivo* and *in vitro* feeding experiments were carried out on days 3, 4 and 5 post rat inoculation with TRUM 273. The fed flies were maintained on clean rabbits until the experiment was stopped six weeks later.

The trypanosome numbers used in membrane feeding were adjusted so that the final concentration suspended in defibrinated clean rat blood was approximately 10^6 trypanosomes/ml. The morphology of these organisms was also noted.

At the end of the experiment all surviving flies were killed and examined for the presence of trypanosome infection. The results of these experiments are shown in Table 7.

Consistent results were obtained from flies which were cooled soon after infective blood meal. The number of flies showing mature salivary gland infections steadily rose with the increase in parasitaemia (and by inference increase in numbers of stumpy trypanosomes). Flies fed on day 3 infections showed about 11% mature infections in both groups of flies *(in vivo* and *in vitro)*. Three times as many infections were obtained for *in vivo* fed flies whereas a fourfold increase in infections was observed among the *in vitro* fed flies at peak parasitaemia, (day 5 infections). The control flies and flies cooled six hours after infective feed showed variable response to infection.

The large number of flies showing mature *T. brucei* infections as a result of these experiments have encouraged us to study the following:

1. ultrastructural and histochemical changes of infected salivary glands.
2. effects of pesticides on infected tsetse
3. role of haemocytes in haemocoelic trypanosome infections
4. attempts to raise susceptible offsprings from trypanosome infected parent *G. morsitans*

These studies are in preliminary stages and therefore will not be reported here. Part of this work was done in the USA (University of Massachusetts,

Amherst) and Walter Reed Army Institute of Research, Washington D.C.)

The Sensitivity of Parasitized *Glossina morsitans* to Toxic Substances. I. Endosulfan

T. K. Godder, S. Y. Patel and P. Onyango

Some of our previous studies (Godder and Patel, Patel and Godder, ICIPE Annual Report, 1979) suggested that a pathological condition exists in the salivary glands of tsetse flies that had mature infections of *T. vivax* or *T. brucei*. Furthermore, previous workers have shown that infected flies exhibit altered metabolic and feeding behaviour. These observations prompted us to predict that a stress condition exists in infected flies and that they would be more sensitive to toxic substances than are uninfected flies. This idea was tested in a trial study and the results are outlined below.

Male *Glossina morsitans* 41–43 days old were injected with topical application of 1 to 25 nanograms of endosulfan (99% purity) on the dorsal thorax. Mortality at each dose was recorded 48 hours after application. Mortality to infected flies was noted only at a dose that gave approximately 50% mortality to uninfected flies, 3 ng/fly (3% dead, n 430). Infected flies of the same age and at the same dose showed 100% mortality (n 16). Furthermore, the death rate was faster in infected flies. Most infected flies were dead within 6 hours of treatment whereas the uninfected flies began to show mortality only after 24 hours.

We are continuing this study and expanding it to include other insecticides as well as some natural plant products. We also intend to assess the effect of age and sex on the susceptibility of infected tsetse flies to toxic substances. The results of such studies should provide enough information to begin to assess the feasibility of a Trypanosome control programme aimed at the selective elimination of infected flies.

The Separation of Subpopulations of Bloodstream Forms of *Trypanosoma brucei*

T. K. Godder and S. Dare

Trypanosomes of the brucei group show a variety of morphological types in the tsetse fly and in the vertebrate host. In the blood of the vertebrate host, the parasites occur as either long slender forms (LSF) or short stumpy forms. The relative proportions of these forms vary from strain to strain and during the parasitemic wave. The LSF are the predominant type during the rise of parasitemia and the SSF increase in number at the peak of parasitemia. There is a body of circumstantial evidence that suggests that SSF represent a state of differentiation that is preadapted for life in the tsetse fly. Definite proof that the SSF are the forms that are infective to the tsetse is lacking because pure populations of SSF have not been available for study. The best studies, to date, have utilized strains of trypanosomes which show 80% SSF at peak parasitemia and none visually detectable two days earlier. We believe that pure populations of forms are necessary before the proper experiment can be done to accurately assess the role of the various morphological types. We have recently performed some experiments to develop chromatographic techniques for analytical separation of trypanosome forms. The successful method for isolating SSF is described below.

The Columns

The separation of SSF from LSF requires two ion exchange columns. The first is an anion exchange column of diethylaminoethyl (DEAE) cellulose equilibrated to pH 8.0 in a phosphate buffered saline, glucose solution (PSG) at an ionic strength of I 0.22. The second column is a cationic exchange column of carboxymethyl (CM) cellulose equilibrated to pH 7.0 in PSG at an ionic strength of I 0.01.

The Technique

Laboratory rats were infected with *Trypanosoma brucei* (EATRO 1969) and blood was collected at first peak of parasitemia. The heparinized blood was applied to the DEAE column and eluted with the high ionic strength PSG at pH 8.0. Under these conditions the blood cells and platelets are retained and the parasites elute (this is the standard Lanham and Godfrey method). The sample was then centrifuged and the parasites resuspended in low ionic strength PSG at pH 7.0. The concentration of parasites was determined, followed by another PSG wash. A known quantity of parasites was applied to the CM cellulose column and eluted with the low ionic strength PSG. The SSF eluted in a sharp peak just after the void volume. The LSF bound tightly to the cellulose and remained bound through five column volumes of eluent. Furthermore, the application of a salt gradient (I 0.01 to I 0.22) eluted through six column volumes, failed to elute the bound LSF.

The results of these experiments is the development of a technique to analytically separate SSF of *T. brucei* from the LSF. This discovery now makes it possible to perform the experiments that should elucidate the function of the SSF. In addition, these results show that the SSF and LSF differ in their surface coat composition. The significance of this difference is unknown at this point but will undoubtably provide an interesting subject for future research.

Fine Structure of Metacyclic *Trypanosoma brucei brucei* Grown in Continuous Culture

M. Nyindo and M. Chimtawi

Introduction

Metacyclic trypanosomes have been cultured *in vitro* from tsetse salivary glands. Morphological studies based on light microscopy showed that the parasites were long, and slender, possessed a free flagellum and the kinetoplast was subterminal. In the course of cultivation electron microscopic studies of the parasites were carried out.

Parasite and Conditions of Cultivation

Rats or mice were inoculated with *T. brucei brucei* EATRO 1969 stock and *Glossina morsitans morsitans* were allowed to feed on the animals at the first parasitemia wave. On days 16 and 60 flies positive for metacyclic trypanosomes were chilled and their salivary glands were placed in tissue culture flasks where embryonic cell from spleen of a cow were maintained at 30°C in RPMI 1640 medium with 20% foetal bovine serum, α-lactalbumin hydrolysate and antibiotics in standard concentrations. Flasks and their contents were incubated at 30°C. When parasites increased in number by 3rd week of incubation they were subcultured. Parasites were processed for electron microscopy when they were 25.0 days or more old in culture.

Electron microscopic procedures

About 10×10^7 parasites were centrifuged at about 1,000 x g for 10 min. The supernatant fluid was discarded. The pellet was fixed in 2.5% glutaraldehyde in 0.5 M sodium cacodylate buffer for 1 – 2 hr at 4°C. Post fixation was done in 1% Osmium tetroxide. Dehydration and embedding were done according to standard procedures. Ultra thin sections ranging from 50 to 90 nm thick were cut and picked up onto uncoated copper grids and stained with uranyl acetate in 50% ethyl alcohol for 30 min and lead citrate for 10 min. Stained sections were examined by the transmission electron microscope, Phillips 201.

Results and Discussion

All parasites possessed an electron dense surface coat measuring from 12.5 nm to 25.0 nm thick beneath which lay the pellicle. Internally peroxisome-like bodies measuring 120 nm to 370 nm diameter and limited by a single membrane were identified. Longitudinal sections of the parasites were filled with at least 8 such bodies (Fig. 13). Many ribosomes were seen and also occasionally a rough endoplasmic reticulum. The kinetoplast was situated subterminally and a chondriome was usually associated with it. The electron dense nature of the interior of the kinetoplast was evident. Many parasites showed an active Golgi apparatus.

Peroxisome-like bodies in bloodstream form *T. rhodesiense* and *T. equiperdum* have been isolated and an enzyme, glycerophosphate oxidase (GPO) has been identified in these bodies. This is one of the enzymes responsible for the catabolism of glucose or other carbohydrate source. It is possible, therefore, that the energy pathway of metacyclic trypanosomes grown *in vitro* is similar to that of bloodstream forms.

The Antigenicity of Metacyclic Forms of *Trypanosoma brucei brucei* Cultured *in Vitro*

C. Powell, M.B.A. Nyindo and J.A. Atema

Metacyclic forms of infectious *T.b. brucei* from the primary antigenic state have been cultured *in vitro* in our laboratory for a period of over one year. The purpose of the following experiments

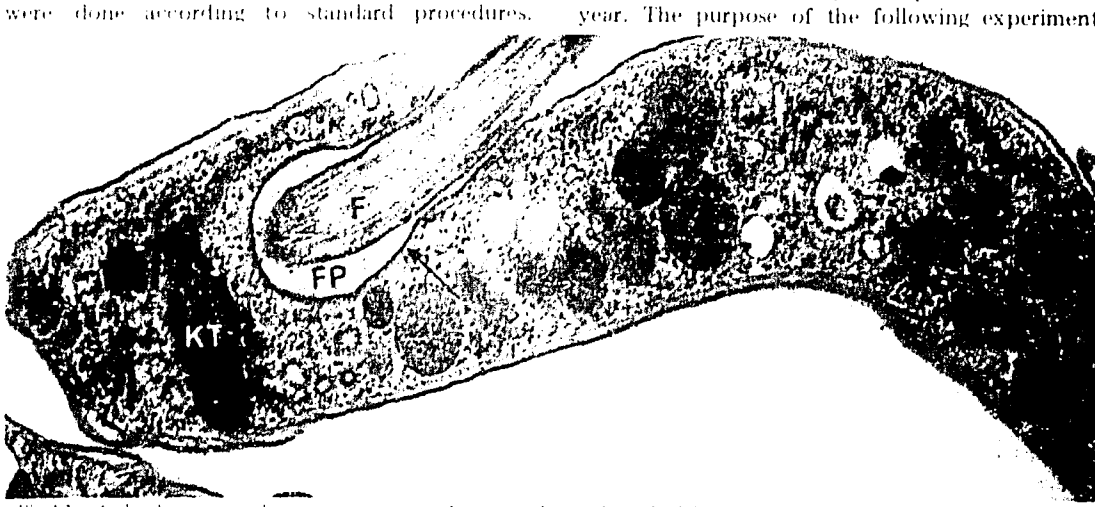

Fig 13: A slender metacyclic trypanosome on day 28.3 of growth in the laboratory. Externally the parasite is covered by a surface coat below which lies the pellicle (arrows). Flagellum (F) and Flagellar pocket (FP) are shown. Internally there are peroxisome – like bodies (PB), endoplasmic reticulum (ER) and a chondrine (CH) is associated with the subterminal Kinetoplast (KT). Magnified 65,000 times.

was to determine the antigenicity of the metacyclic forms of *T. b. brucei*. The E.L.I.S.A (Enzyme Linked Immuno Sorbant Assay) technique was used to measure the antigenicity.

EATRO *T. b. brucei* 1969, grown on bovine embryonic splenic cells and EATRO *T. b. brucei* 999, grown in the same feeder layer or in the presence of pupa cells of *Glossina m. morsitans*, all grown in continuous culture, were used.

Tables 8 – 10 describe the O.D. (405 mu) ml of antibodies to the homogenate (anti whole parasite) of EATRO *T. b. brucei* 1969 and EATRO *T. b. brucei* 999 as against the respective metacyclic antigens. The antibodies were formed utilizing Freund's adjuvant (the first injection with the complete and further 1 – 2 injections with the incomplete.)

Table 8

Antibody Titers of Anti Homogenates to the Metacyclic (day 2.39 – 850) and Blood Stream Forms.

Antigen – EATRO *T. b. brucei* 1969 grown on bovine splenic cells.

ANTIBODY	ANTIBODY TITER	
	IgG	IgM
H_F 1969	0.3282	0.908
H_{INF} 1969	0.0789	0.4188
H_F 999 pupa	0.7204	0.2370
H_{INF} 999 pupa	0.2870	0.1905
H_{INF} 999 spleen	0.2489	0.0581

H_F – anti homogenate made in Freund's adjuvant (complete/incomplete).

H_{INF} – anti homogenate made in response to infection in the first peak of parasitemia from metacyclic forms in O.D. (405 mu)/mg protein/ml

Table 9

Antibody Titers of Anti-Homogenate to the Metacyclic and Bloodstream Forms.

Antigen – EATRO *T. b. brucei* 999 grown on bovine splenic cells.

ANTIBODY	ANTIBODY TITER	
	IgG	IgG
H_{INF} 999 (spleen)	0.2008	0.1353
H_F 999 (pupa)	0.5057	0.2166
H_{INF} 999 (pupa x)	0.0212	0.0999
H_F 1969 (spleen)	0.3838	0.1722
H_{INF} 1969	0.0789	0.0623

spleen – antibodies from metacyclic forms grown on bovine splenic cells

pupa – antibodies from metacyclic forms grown in the presence of pupa cells of *Glossina m. morsitans*.

pupa x – bloodstream forms from the first peak of parasitemia in rats obtained from metacyclic forms grown in the presence of pupa cells of *Glossina m. morsitans*.

Rest of legend as for Table 8.

Table 10

Antibody Titers of Anti-Homogenate to the Metacyclic and Bloodstream Forms

ANTIBODY	ANTIBODY TITER		ANTIGEN
	IgG	IgM	
H_F	.849	.247	Metacyclic
H_F	.875	.771	Bloodstream
H_{INF}	.117	.258	Metacyclic
H_{INF}	.523	.118	Bloodstream

Antigen — EATRO *T.B. brucei* 999 grown on bovine spleenic cells — Metacyclic.

EATRO *T.b. brucei* 999 at first peak of parasitemia grown in a rat. — Bloodstream.

Rest of legend as for Table 8

When reacted against the metacyclic homogenate antigens, antibodies formed with adjuvant to the metacyclic forms demonstrated a higher antibody titer than antibodies formed by the first peak of infection. This difference in the antigenicity could be an indication of a change from the metacyclic form to the bloodstream form.

The anti-homogenate of EATRO *T.b. brucei* 999 grown on pupa cells gave higher antibody titers. Which part of these increased titers are immunoprotective antibodies, needs still to be investigated.

In order to determine the antigenic stability of the metacyclic forms grown in culture that were used to make these antibodies, the antibody titer (by use of ELISA) of antigens from different periods of cultivation, was analyzed (detailed in Table 11). No significant difference in antigenic titer after an approximate one year period, (between day 239–258 to days 680–722) was observed, suggesting that the metacyclic forms grown in continuous culture are in a stable antigenic state.

Immunoprotective Studies on the Metacyclic Forms of *Trypanosoma brucei brucei*

C. Powell, M.B.A. Nyindo and J.A. Atema

One major feature that is characteristic of trypanosomes is that no trypanosomal immunogen would give protection against any heterologous strain or species in terms of complete immunoprotection or even in terms of increased pre-patent period (time between challenge and infection) or lowered parasitemia.

Previous results in Zambia showed that a subcellular fraction, "fraction 3" of *T.b. rhodesiense* could induce immunoprotection across strains and species of trypanosomes, (C. Powell and L.T. Mathaba, Med. J. Zambia, 1978 12,3, pp 67–9).

Further to these findings, we assayed the antibody production formed by this fraction from the metacyclic forms of *T.b. brucei* grown *in vitro* in continuous culture. Table 12 shows that the

Table 11

A comparison of Antigenicity of EATRO *T.b. brucei* 1969, days 238 – 259 to days 680 – 722

AGE OF ANTIGEN	ANTIBODY TITER	
	IgG	IgM
day 238 – 259	0.30	0.09
day 680 – 722	0.25	0.08

Antibody H_F 1969 (day 238 – 259) and (day 580 – 722) in O.D. (405 mu)/mg protein/ml.

Rest of legend as in Table 8.

Table 12

Antibody Titer to the Immuno Protective Fraction (IPF) Compared to Antibody Titer to Whole Homogenate (H).

Antigen EATRO *T b brucei* 999 grown on bovine cells

ANTIBODY	ANTIBODY TITER	
	IgG	IgM
H 1969	0.3282	0.0908
IPF 1969	0.4660	0.0462
H 999	0.7204	0.2374
IPF 999	0.8218	0.2739

Antigen EATRO *T b brucei* 999 grown on bovine cells.

ANTIBODY	ANTIBODY TITER	
	IgG	IgM
H 999	0.5057	0.2166
IPF 999	0.9809	0.3528
H 1969	0.3838	0.1722
IPF 1969	0.6055	0.1449

in O.D. (405 mu)/mg protein/ml

H	Antibodies to the respective homogenate.
IPF	Antibodies to the respective IPF
1969	EATRO *T b brucei* 1969 grown on bovine spleenic cells.
999	EATRO *T b brucei* 999 grown in the presence of pupa cells of *Glossina m. morsitans*.

In Freund's complete incomplete — 1st injection with complete adjuvant and subsequent injections with incomplete adjuvant

Immunoprotective Fraction (IPF), "fraction 3" has a higher antigenicity than any respective homogenate that it is compared with. EATRO *T b brucei* 999 had a higher antigenicity than EATRO *T b brucei* 1969. *T b brucei* had an even higher antigenicity count when grown in the presence of pupa cells of *Glossina m. morsitans*. Comparison of antigenicity and immunoprotection must be carried out.

MEDICAL VECTORS RESEARCH PROGRAMME

Programme Leader

Dr. R. Subra (1978)

Research Advisor

Professor J. Mouchet (1975)

Research Staff

Dr. A.W.R. McCrae (1977) Senior Research Scientist
Dr. J.M. Mutinga (1979) Senior Research Scientist
Dr. W.A. Otieno (1978) Research Scientist
Dr. F.W. Mosha (1978) Research Scientist
Dr. G.P. Kariya (1978) Postdoctoral Research Fellow
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Dr. M.O. Odindo (1980) Postdoctoral Research Fellow
Miss L.M. Oketch (1978) Graduate Research Scholar
Miss L.W. Irungu (1978) Graduate Research Scholar
Dr. J.O. Otolo (1978) Graduate Research Scholar
Miss D.M. Sabwa (1979) Graduate Research Scholar
Mr. C.M. Mutero (1979) Research Trainee, University of Nairobi
Mr. S. Muti (1978) Junior Technician
Mr. P.M. Mwamisi (1978) Junior Technician
Mr. M.C. Ngumah (1980) Junior Technician
Mr. R.C. Odhiambo (1980) Junior Technician
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Mr. P. Amutalla (1979) Technical Assistant
Mr. E. Mkuzi (1976) Technical Assistant
Mr. J. Mwandandu (1971) Technical Assistant/Driver

Introduction

Controlling vector borne diseases is one of the major components of rural development in tropical Africa. Since insecticides cannot be the only answer to this problem research on medical vectors at ICIPE which includes mosquitoes and sandflies has been oriented towards the development of alternative control methods which require basic knowledge on both immature and adult stages of the vectors. Not only the vectors themselves but also their natural enemies and other regulatory factors are being looked into.

In 1980 the Medical Vectors Programme consolidated its on going activities, achieving a number of positive findings and developing several new lines of research. The project takes full advantage of the excellent field facilities afforded by the Kenya environmental situation.

Anopheline Aquatic Ecology

A.W.R. McCrae

The *Anopheles gambiae* species complex includes two of the world's most efficient malaria vectors which cannot be controlled except by the most intensive use of currently available measures. This and the recently proven emergence of 4-amodiaquine(chloroquine) resistant *falciparum* malaria in Eastern Africa underline the urgency for the international research community to intensify exploration of new avenues with a view to improved control. The present project, which commenced at the ICIPE Coastal Field Station in 1978, has concentrated on two barely-touched topics, namely oviposition and aquatic population regulation of the freshwater species of the *gambiae* complex.

On oviposition the most important findings of 1980 are, firstly, that whereas freshwater *gambiae* oviposit from a sedentary posture when presented with a pale-coloured target (i.e. under conditions facilitating most detailed observations), dark targets elicit a distinctive oviposition flight. At simulated natural breeding sites the female mosquito orients by facing the bare mud-water edge while ovipositing from rapid zigzag flight onto the water surface. Secondly, a long series of discrimination experiments indicate that natural breeding site waters contain a weak arrestant factor which is not derived form the mosquitoes' own aquatic stages, either eggs, larvae or pupae. These and other findings pave the way to development of a field sampling method based on interception of the ovipositing female, on which field trials have commenced.

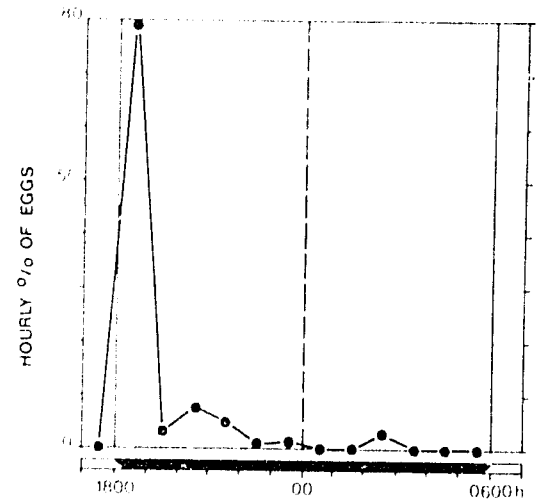

Fig 1: Circadian pattern of oviposition by hypergravid female freshwater *A. gambiae* having been held after ovary maturation for a further 24 h without access to water: 2 nights; 6,098 eggs.

Studies have been concluded on the circadian periodicity of oviposition, showing hypergravid females to have an extremely marked post-sunset peak of activity (Fig. 1), thus indicating in combination with last year's results that the timing of oviposition is not controlled by an endogenous rhythm but is a function of the time elapsing after the blood-meal. This is confirmed by a relationship with temperature as shown in Fig. 2. These results would help to decide when to conduct field studies on oviposition with greatest efficiency.

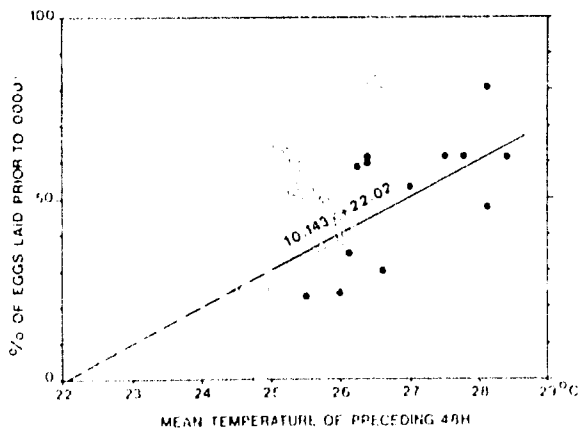

Fig. 2. The proportions of nightly totals of eggs laid prior to solar midnight by normally gravid females of freshwater *A. gambiae* on 14 nights, plotted against the mean ambient temperature of the preceding 48 hours. The regression line and coefficients are shown, with a theoretical intercept of zero eggs pre-midnight falling at 27.0°C. However, the accepted switch from a 2-day to a 3-day gonotrophic cycle in *A. gambiae* is at, or approximately 23.5°C (Fig. 2).

Work on egg darkening has provided a new technique for distinguishing between freshwater and seawater-breeding species of the *gambiae* complex, simpler and more rapid than the standard method currently in use. Eggs laid by *A. merus* darken normally in, up to (and often above) 90% sea salinity, whereas those of freshwater species fail to darken above 55%. Thus, darkening of eggs laid directly into water of 70% sea salinity is diagnostic. Provided the mosquitoes are held under optimal conditions until fully gravid or preferably hypergravid, differential species specific adult mortality arising from excessive exposure to the saline water will not skew the results.

Studies continue on aquatic population regulation by means of regular monitoring and by field and laboratory experiments. The phenomenon of very marked pupal deficits has been demonstrated many times in a range of different habitats, especially evident in the more permanent waters. Two hypotheses to account for this remain open; one, that predation is greatly intensified as pupation approaches and (perhaps) proceeds, and the other, that a surface effect exists which inhibits pupation or which kills pupae, and which is dispersed by disturbance such as heavy rain or the action of sampling.

The two hypotheses may not be mutually exclusive. Evidence of severe predation is often seen when successive anopheline larval stages at an overall low density fall steeply to zero by the 3rd or 4th instar. Enhanced larval survival and the appearance of pupae tend to become apparent when both nymphal and adult notonectid densities are low. These notonectids (*Anisops* spp.) show cycles of breeding commencing when water levels rise with heavy rain and flood into marginal vegetation; their eggs are inserted into plant tissues. Resultant nymphs become adults within a few weeks, but without further rain these adults accumulate. Relatively light rain may then induce adult dispersal flights, reducing densities in the more permanent waters. Then, until water levels rise again and nymphs reappear, predation pressure appears to be relaxed (Fig. 3). Difficulty of interpretation arises however in that it is the notonectid nymphs rather than the adults which forage in the anopheline shallow water microhabitat; indeed, the data are so far insufficiently clear to show whether or not both these potential prey and predator subpopulations are merely responding, directly or indirectly, to the common factor, rainfall. We have nevertheless found that the final anopheline larval instar is of considerably longer mean duration than that of any of the preceding instars, or of pupae. Furthermore, laboratory results indicate that high adult notonectid densities may slightly retard anopheline larval development without direct contact, thus potentially enhancing predator efficiency. However, no such effect of notonectids on pre-pupal or pupal mortality was apparent from experimental results.

Pupae have tended to occur in situations of moderately low larval density, when the decline in numbers of 4th instars to pupae compared with the decline of 3rds to 4ths has been at an overall average of approximately 7-fold. Such a rate of decline could be explained by simple predation in view of the long 4th instar duration and of the largest larvae perhaps being the easiest targets as prey. However, at highest larval densities this decline averages at some 25-fold, suggesting that a crowding factor may be responsible. Whatever the cause, it is abundantly clear that a very powerful brake on adult production exists at or just before the pupal stage, which we do not yet understand. To solve this problem remains our highest priority.

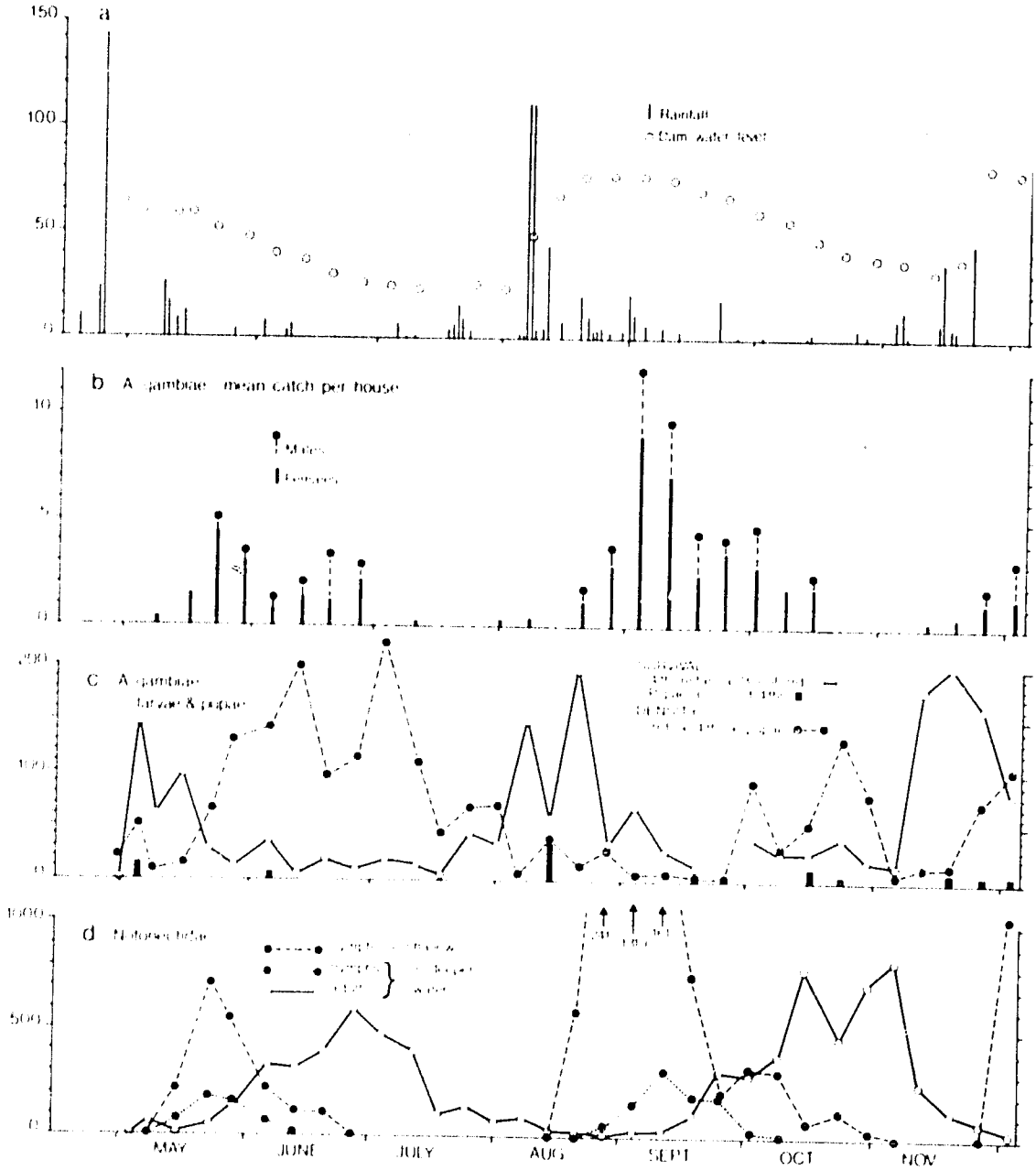

Fig. 3: Monitoring data, Simakeni, 15 km W of Mombasa. a: Daily rainfall and weekly dam water level. b: Adult *A. gambiae* densities per house from weekly hand catches some 400-500m from the dam. This indicates potential egg input rather than adult output from the dam. c: *A. gambiae* aquatic stages. Late instar larval and pupal densities, survival rates, from weekly sampling from 7 points around the dam margins. d: Notonectidae (*Anisops spp.*). Nymphs strained from 0.125 m^3 of water from 2 of the *Anophelines* sampling sites in the shallows of the dam, and adults and nymphs sweep netted from 0.8m^3 of deeper water. For direct comparison, nymphs from the shallows should be 6.4 times more numerous than shown. Other predators in the shallows, e.g. Pleidae or small Dytiscidae, showed similar incidence to notonectid nymphs.
Note that anopheline high larval survival and to a lesser extent pupal incidence tended to occur during low notonectid density. Small notonectid nymphs predominating early in nymphal upsurges would have had slight predator impact on the larger anopheline aquatic stages.

Ecological Studies on *Anopheles gambiae* Sibling Species

F.W. Mosha

Longitudinal studies on *Anopheles gambiae* sibling species which commenced in August 1978 in Jimbo village on the south Kenya coast were continued. During this period, attempts were made to compare the relative densities, biting cycles and malaria and filarial infection rates among the individual *An. gambiae* sibling species occurring in this area. However, this information cannot be presented in this report since most of the chromosome preparations which were made for these sibling species have not been read. Field and laboratory observations were also carried out to determine the influence of salinity on *An. merus* larval development and population dynamics.

Mosquitoes were collected by human bait method from outdoor and indoor houses in Jimbo village. Out of 1945 *An. gambiae s.l.* mosquitoes collected between December 1979 and November 1980, 516 were processed for chromosome preparations while most of those remaining were separated into *An. merus* and freshwater breeding *An. gambiae* by salt tolerance test. Dissections for sporozoites and filarial worms were carried out in the field. Fortnightly samples of water from a semi permanent pond were analysed for salinity by titrating with silver nitrate solution.

Newly hatched larvae of *An. merus* were also reared in different dilutions of sea water and its component individual chemical salts as listed in Table 1. The LC50 of these salts on the *An. merus* larvae was estimated in the same manner as for larvicides.

Fig. 4 shows that there was wide fluctuation in rainfall between August 1979 and November 1980 (0–105 mm). These fluctuations were also reflected in the salinity variations in Jimbo pond water. *An. merus* densities were directly affected by changes in the salinity of the breeding sites. The peak *An. merus* density period coincided with salinities of 35.5% and 30.3% sea water in the semi-permanent pond during 1980, and with a salinity of 48.2% sea water during 1979. Anopheline larvae were also most abundant in the pond at these salinity levels.

Laboratory studies showed that *An. merus* larvae were capable of undergoing full development in sea water dilutions ranging from 0% to 100% sea water. However, the larvae were found to show some seasonal variations in their tolerance to sea water. Development of *An. merus* larvae in pure sea water was only observed in two replicate tests (out of 6) of 25 larvae each carried out in June 1980, a period when water salinity in the semi-permanent breeding site was 52.0% sea water. In the other replicate tests carried out in November 1979 and September 1980 when the respective salinity in the breeding site was 48.2% and 30.3% sea water, the first stage larvae of *An. merus* died within two days of exposure.

LC50 (median lethal concentration) of several salt constituents of sea water (Table 1) were carried out for first stage larvae, newly hatched from 157 egg batches laid by *An. merus* females collected from Jimbo village. Four replicate tests of 25 larvae each were run for each of the salts, using 50 mls of the solution for the concentrations 0, 0.63, 1.25, 2.5, 5, 10, 20, 40 and 80 gm salt/litre.

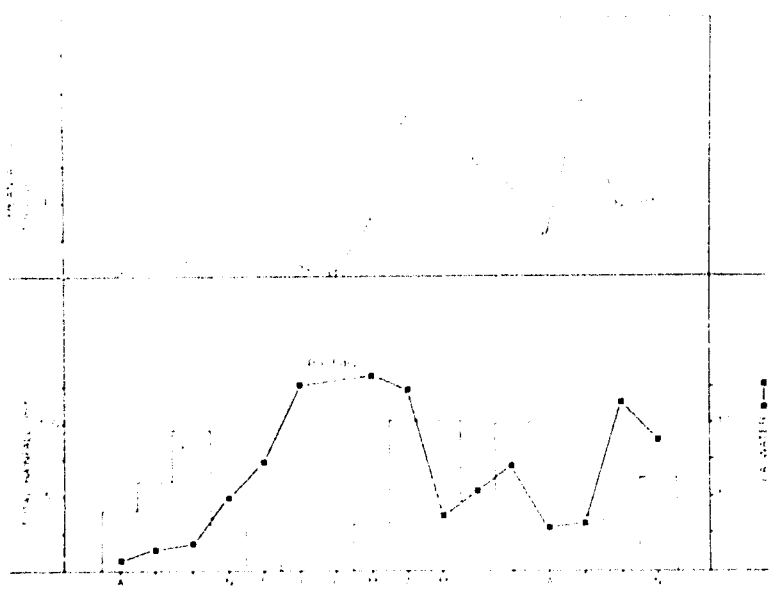

Fig. 4 Monthly fluctuations of rainfall, breeding water salinity (expressed as percent sea water) and mean *An. merus* bites per person per night in Jimbo village between August 1979 and November 1980.

Table 1 Tolerance and development of *An. merus* larvae in different chemical salts found in sea water

SALT	GRAMS PER LITRE	
	LC_{50}	Maximum conc for full development
Sodium chloride	32.5	20.0
Potassium chloride	11.5	10.0
Magnesium chloride	13.2	10.0
Calcium chloride	50.0	10.0
Sodium sulphate	122.0	20.0
Magnesium sulphate	50.0	26.0
Sodium carbonate	12.0	2.5
Potassium carbonate	19.5	5.0

All the larvae, surviving the LC50 test period (two hours) for the various salts were left to complete their development. Table 1 shows the LC50 of the eight chemical salts to the first stage larvae of *An. merus*. The highest LC50 was observed in *An. merus* larvae exposed to sodium sulphate, and least in those exposed to potassium carbonate. Maximum salt concentrations of *An. merus* larvae ranged from 2.5 to 20.0 g salt/litre. These results suggest that chemical salts other than sodium chloride present in sea water may play a significant role in influencing the development of An. merus larvae.

Biting Activity and Resting Behaviour of *An. merus* and Fresh Water Breeding *An. gambiae* on the Kenya Coast

C.M. Mutero

Ecological studies on *An. merus* and fresh water breeding *An. gambiae* were conducted in two villages, Jimbo and Jego, on the Kenya South Coast between February 1979 and May 1980. The main aim was to compare the biting activity and resting behaviour of these *An. gambiae* sibling species. *An. merus* was the most abundant sibling species in Jimbo and constituted 86.0% of the 1,387 female *An. gambiae s.l.* that were identified by salinity tolerance test. In Jego, fresh water *An. gambiae* sibling species were predominant and comprised 98.5% female *An. gambiae s.l.* tested.

The biting cycle of *An. merus* outdoors and indoors gradually rose from 1800 hours and attained a peak between 2300 hours and 0100 hours and then lowered towards 0600 hours (Fig. 5). The biting patterns of fresh water *An. gambiae* (in Jego village) had steeper slopes than for *An. merus* and showed two peaks outdoors, one between 2200 hours and 2300 hours, the other between 2400 hours and 0100 hours. The peak of the indoor biting cycle occurred between 0100 hours and 0200 hours. Overall, the outdoor and indoor biting cylces of *An. merus* were more similar and less erratic than those of fresh water *An. gambiae*.

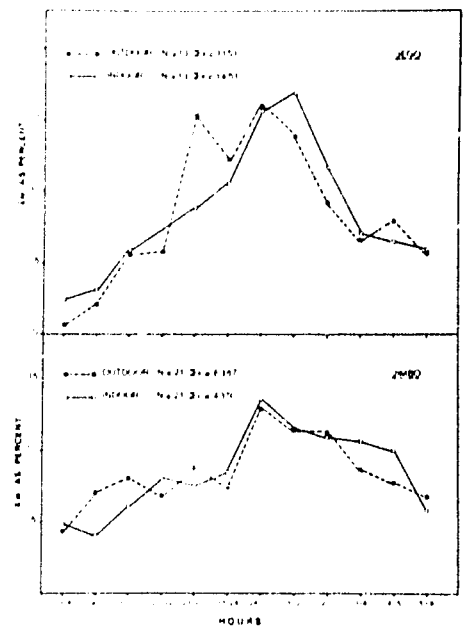

Fig 5 Outdoor and indoor biting cycles of *An. gambiae s.l.* from Jimbo and Jego (N denotes the number of full 12 hour human bait-catches considered.)

The *An. merus* population in Jimbo was significantly exophagic (P 0.01) and in a total of 14,334 female *An. gambiae s.l.* collected in 66 full night catches 60.2% were caught biting outdoors. In Jego, the population of fresh water *An. gambiae* between March and May 1979 was significantly endophagic (P 0.01) and out of 2,284 females collected in 9 full night catches, an average of 67.7% were caught biting indoors. A reversed situation with significantly excessive bting occurring outdoors (P 0.01) was observed in Jego between July and October 1979. During this period the proportion biting outdoors averaged 69.2 for 6 full night catches in which a total of 799 females were collected.

The *An. menus* population in Jimbo was deliberately exophilic and made up about 90% of the female *An. gambiae s.l.* collected while resting indoor, sites such as heaps of coconut husks, upturned roots of coconut trees, and fallen coconut leaves. *An. menus* formed only about 36.6% of the females collected resting inside houses in Jimbo. Out of 727 female *An. gambiae s.l.* collected resting outdoors in Jimbo, the proportions of unfed, fed, semi-gravid and gravid were 64%, 27.4%, 4.8% and 3.7% respectively. Precipitin tests of 105 blood smears prepared from freshly fed female *An. gambiae s.l.* caught outdoors in the village showed a human blood index (HBI) of 59.8%. In Jego, the *An. gambiae s.l.* population was markedly endophilic. A total of 493 female *An. gambiae s.l.* were caught resting inside houses and 99.4% of the 264 that were identified by salinity tolerance test comprised of fresh water *An. gambiae*. During several searches in a period of over three months, only four female *An. gambiae s.l.* were caught resting outdoors in Jego. These were found inside artificial box shelters and the two that were identified proved to be fresh water *A. gambiae*. Natural outdoor resting sites of fresh water *An. gambiae* were not located.

Competition Studies Between Culex *pipiens quinquefasciatus* (=Fatigans) and *Culex cinereus*

R. Subra

Studies in 1979 had shown, both in the laboratory and in the field that *C.p. quinquefasciatus*, a vector of Bancroftian filariasis, was unable to maintain its premaginal populations in breeding places which were colonized by *C. cinereus*, a non man-biting mosquito.

The work carried out in 1980 had 2 objectives: 1. to check if the replacement of *C.p. quinquefasciatus* by *C. cinereus* at the premaginal level was a general phenomenon, i.e. if it could again be observed in 1980 and if this was reflected in the adult densities of *C.p. quinquefasciatus* in the study area, 2. to check if the selective distribution of *C. cinereus* in some breeding places could be explained by some adverse aspects of human behaviour, namely the introduction into breeding places of used water containing detergents.

These studies were done in the Rabai area 20 km north of Mombasa. In that area the 2 mosquito species are developing in latrines where the rising water table provides them with suitable conditions for breeding. Studies on objective No. 1 started in May 1980. *C.p. quinquefasciatus* and *C. cinereus* premaginal population dynamics were studied by monitoring the adult production of two latrines about 100 m from each other in a small village located on the slope of a hill. They had been built at different levels and thus were called "upper" and "lower" breeding place. Each of them had two holes. An exit trap was placed over one hole, and the other was left open to allow free access for entry of gravid females. Twenty four hour catches took place once every 3 days, commencing at 8.00 a.m. Only males of the 2 species were considered as it had been observed in 1979 that the greatest majority of them were freshly hatched, thus giving a satisfactory picture of the breeding place productivity. *C.p. quinquefasciatus* adult densities were assessed by hand collections of indoor resting mosquitoes in another small village of the Rabai area. This specific work was part of a routine monitoring on adult mosquito population carried out once weekly by Dr. A.W.R. McCrae.

Studies on objective No. 2 started in November 1980, when *C. cinereus* was the only species to colonize the two breeding places. A traditional bathroom was built beside the lower latrine. Some of the men from the village were requested to utilize it and a pipe system was devised so that used water from the bath was poured into the latrine together with a daily constant amount of detergent prepared for that purpose. This detergent would have seeped downhill in ground water without contaminating the upper latrine, which was kept as a control. The results of this experiment were also assessed by monitoring newly emerged males.

Results of both these studies are shown in Figure 6.

The lower breeding place started to produce *C.p. quinquefasciatus* by the end of May, a few weeks after the first wave of heavy rains. A second wave of rain at the beginning of August flooded the upper breeding place where *C.p. quinquefasciatus* was again the first species to be recorded. Peak catches of this species in both breeding places occurred on the same day towards the end of August. At first considerable densities of *C. cinereus* were observed at about the same period in both breeding places. The absence of this species in the lower breeding place during the preceding 2 months cannot be satisfactorily explained. Both species shared the two breeding places for a few weeks after which an increase of *C. cinereus* was accompanied by a short decrease, followed by the disappearance of *C.p. quinquefasciatus* by mid-September. Until the introduction of detergents to the lower breeding place in early November, *C. cinereus* production remained very high.

C.p. quinquefasciatus adult densities are expressed in Figure 6 as the number of males and females caught per house. The peak density observed in June could be explained by the rising water table in some latrines at the end of May, as observed in the lower breeding place. Numbers probably decreased in July because of inadequate rains in June and consequent lowering of the water table. The second wave of rains which

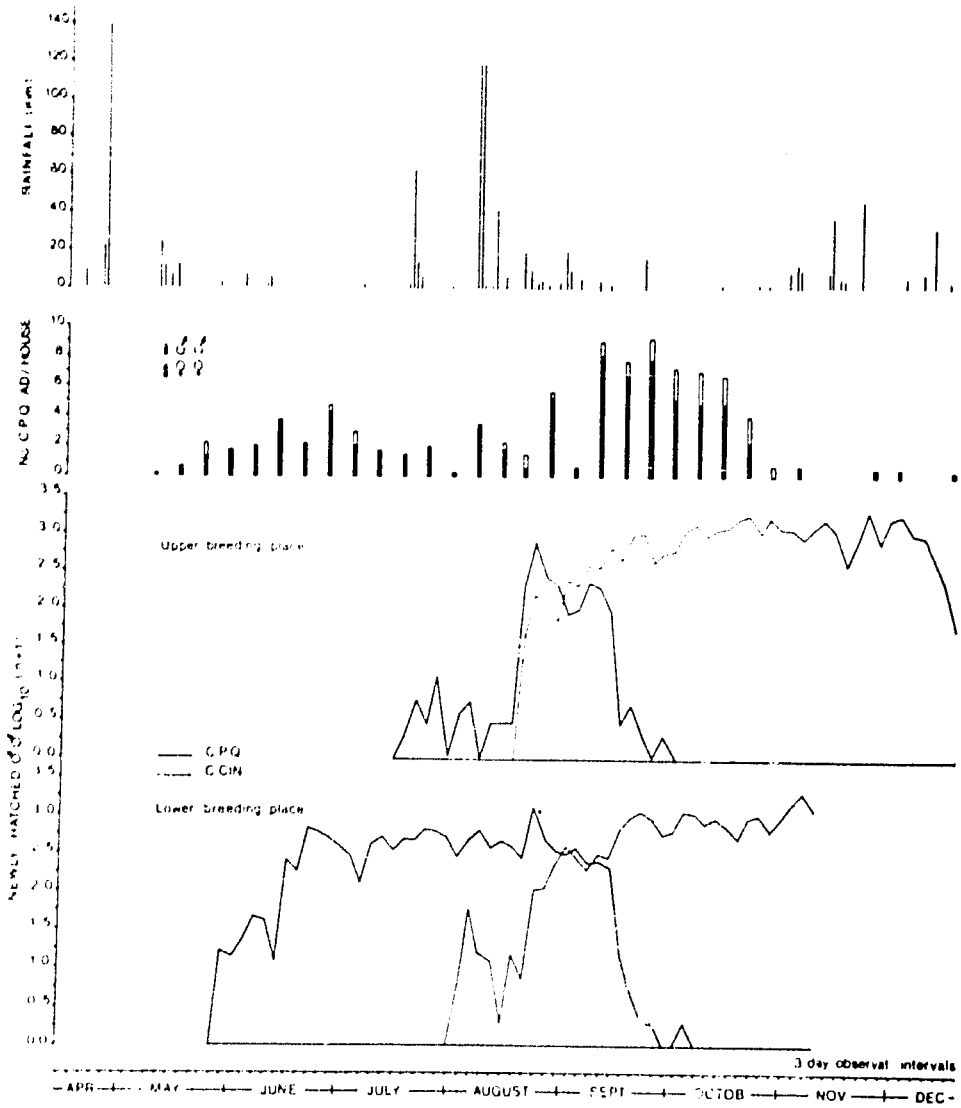

Fig 6. Field evidence of interaction between *C. p. quinquefasciatus* and *C. cinereus* and the effects of detergents in breeding sites.

flooded the majority of latrines, as seen in the upper breeding place was followed by a sharp increase of densities which peaked in September. From then the number decreased through October to nil in early November. It is noteworthy that during this period *C.p. quinquefasciatus* disappeared from breeding places which were still flooded but only colonized by *C. cinereus*. Thus there is a correlation between the observations of breeding place productivity and *C.p. quinquefasciatus* densities in houses.

The introduction of detergents into the lower breeding place did not immediately affect the production of *C. cinereus* adults, which fell very sharply 1–2 weeks later. At this time sampling of the preimaginal stages from this site showed only old larvae and few pupae, while young larvae were completely absent. From the control breeding site, adult production remained high with all preimaginal stages including early instar larvae still present.

The observations in 1980 have shown that the replacement of *C.p.q.* by *C. cinereus* was a general phenomenon in the Rabai area, confirmed by the low densities of *C.p. quinquefasciatus* when *C. cinereus* was the only species present in the breeding sites. *C. cinereus* is not able to maintain itself in the sites in which used water with detergents is poured daily. The next step in this research will be on the possible recolonization by *C.p. quinquefasciatus* of breeding sites which have been freed of *C. cinereus*.

General Survey of Various Mosquito Habitats in Kenya

L.M. Oketch

The immature stages of different breeding habitats of the Kenya Coast and the Nairobi area were sampled to determine the mosquito fauna. These habitats were treeholes, ponds, dams and rockholes. Work was concentrated on the immatures because it is by making individual rearings from the immatures that a definite association of larval, pupal and adult stages is possible, and reared material is excellent for taxonomic work and for reference collections.

Individual rearings: Some of the fourth instar larvae sampled from the breeding sites were isolated into plastic cups with a little water, supplemented with baby food (farex) and allowed to develop until adult stage. Both larval and papal skins were preserved. The adult was allowed to harden before being killed and mounted. The rest of the fourth instar larvae were preserved in Lactophenol. The younger instars were left in their breeding water until they reached fourth stage, whence they were isolated. Larvae, larval and pupal skins and adults were mounted and male genitalia prepared according to Edwards technique.

Three forests were sampled for tree-hole breeders, one at the Kenya Coast (Muhaka Forest), 2 in Nairobi area (Ngong and Karura Forests).

At Muhaka Forest 15 known treeholes; at Ngong 12 and at Karura Forest 7, all of man-reaching heights, were sampled with bottle aspirators. Each tree in Ngong and Karura forests had bamboo traps attached to them. The bamboo sections were left for two weeks *in situ* to be emptied and refilled. These sections were sampled only in the dry season. All the treeholes studied and the bamboo sections were at man-reaching heights, therefore, species that breed at greater heights were not sampled.

The period of study being limited to a part of the dry season, and at the onset of the rains, the succession of species was not revealed, some species may also have been missed out, especially those that appear during the rains, or towards the end of the rains.

Table 2 shows the species collected in these different breeding places.

Three species, *Aedes aegypti*, *Ae. hawortbi* and *Culex nebulosus* were the only species found common in the three forests. *Ae. soleatus*, *Ae. heischi*, *Ae. apicoargenteus*, *Ae. fulgens*, *Ae. marshalli*, *C. horridus* and *Anopheles gambiae s.l.*

Table 2: Treehole mosquitoes recorded at Kenya Coast (Muhaka) and Nairobi (Ngong and Karura) Forests, Collection Method: T = Treehole B = Bamboo Trap. Stages available in the Reference Collection L = larval skin; P = Papae, A = adult

Genus and Subgenus	Muhaka Forest 39° 32 'E, 4° 19 'S	Ngong Forest	Karura Forest	Tree Types containing the Treeholes investigated N = Nairobi area, C = coast
Aedes (*stegomyia*)	*Soleatus* Edw (T: L, P)		*K iensis* van (T: L, P, A)	*Rutacea tetcatrichocarpa* (N) (Engl.) •
	heischi Someren (T: L, P, A)		*schoreri* Edw (B: L, P, A)	*oleaceae olea africana* (Mill)
	aegypti L (T: L, P, A)	*aegypti* (T: B, L, P, A)	*aegypti* (T, B: L, P, A)	*Thretia cymosa* Thorn.
	apicoargenteus Theo			*Teclea simplicifolia* (Engl) Verdoon.
(*Finlaya*)	*fulgens* Edw (T: L, P, A)	*Pulchrithorax* Edw (T, B: A, P, A)	*Pulchrithorax* (T, B: L, P, A)	*Neuctonia buchanenii* (Bak)
(*Aedimorphus*)	*hawortbi* Edw (T: L, P, A)	*hawortbi* (T: L, P, A)	*hawortbi* (T, B: L, P, A)	*Dtrypetes natalensis* (Haru) Hutch. C
	Michaehkati Van Som (T: L, P, A)	*ngong* Van Someren (T: L, P, A)	*ngong* van Someren (T, B: L, P, A)	*Brachystegia speciformis* C
	Marshalli Theo (T: L, A)	*Pseudotarsalis* (B: L, P, A)		*Craibia brevicaudata* (Vatke) Dunn. *subsp. brevicaudata* C.
Culex (*Culiciomyia*)	*nebulosus* Theo (T: L, P, A)	*nebulosus* (T: L, P, A)	*nebulosus* (B: L, P, A)	*Mkilua fragrans* Verdcourt
	horridus Edw (T: L, P, A)			*Annonaceae*
Anopheles	*gambiae* s.l* (T: P, A)			

Table 3 Rockhole Container, Dam and Pond Breeders

Rockhole Breeders from Kombeni River (Raban)	Brackish Breeders, Jimbo (South Coast) 39° 12 E and 4° 0s 'S	Fresh Water Dam Breeders in Kabete	Rubbish Can Breeders Breeders in Chiromo
Culex (culex) pipiens quinquefasciatus Say	*Ae. (Aedimorphus) albocephalus* Theo	*C. p. quinquefasciatus* Say	*Ae. (Stegomyia) aegypti*, L
C. (culex) annulioris Theo	*Ae. (Aedimorphus) tessellatus* van Someren	*C. (culex) trifilatus* Edw	*C. nebulosus* Theo.
C. (culex) nebulosus Theo	*Ae. (Aedimorphus) argenteopunctatus* Theo	*C. nebulosus*, Theo.	*C. p. quinquefasciatus*, Say
C. (culex) simpsoni Theo	*Ae. (Aedes) pembaensis* Theo	*C. annulioris* (Theo)	
Aedes (Stegomyia) vittatus Bigot	*Ae. vittatus* Bigot		
Anopheles gambiae s.l	*Ae. (Banksinella) albothoracis* Theo*		
Anopheles spp	*C. (Neoculex) wigglesworthi* EdW*		
	C. (culex) argenteopunctatus vertrillon*		
	C. (culex) tritaeniorhynchus Giles*		
	C. (culex) vansoni wiedemann		
	C. (culex) simpsoni Theo.*		
	C. quinquefasciatus Say*		
	Eretmapodites quinquevittatus Theo.*		
	Mansonia (Coelepiomyia) metahelmicta Theo*		

were recorded only at the coast. It appears that the coastal zone has more species abundance than the upcountry forests. Few larvae of *A. gambiae s.l.* were recorded once from a tree-hole that had fallen and was close to ground level.

The most abundant species at the coastal forest were: *C. nebulosus*, *Ae. fulgens*, *Ae. haworthi* and *Ae. aegypti*. Upcountry the most abundant species were *C. nebulosus*, *Ae. pulchrithorax* and *Ae. ugong*.

In addition to tree-hole breeders, samples were taken from rock holes, containers, dams and brackish ponds (Table 3). Kombeni river, north of Mombasa was the rockhole sampling site in the dry season when the river dries up leaving rock bed with holes. *Aedes vittatus* was the most abundant species recorded in this breeding site. Three species were found to be abundant in cans and 4 in a moderately polluted dam in Kabete.

Brackish and Pond Breeders: Sampling was done in a small village (Jimbo) at the Kenya south coast. Salinity in this semi-permanent pond varies with different seasons, salinity being higher in the dry season. Table 3 shows the species sampled in this pond. In addition net traps used to sample resting adults were set near this pond. The species shown in asterik in Table 3 were collected in these traps. Since these species from traps were not collected from the pond as immature, they may be breeding there or utilizing other breeding places. One apparent new species has been found in this area and arrangements are underway to describe and give it a name.

At the beginning of October 1980, work was started in Jimbo and Vanga area on brackish water breeders. In this work, it is hoped that the different species breeding in brackish water will be identified using morphology and other experimental taxonomic methods. Already attempts have been made to study the sounds produced by the mosquito species from this area using a Bruel and Kjaer ¼ inch condenser microphone and microphone amplifier tape recordings were done on a B & K instrumentation tape recorder. In later stages it is hoped that the flight sounds will be analysed and possibly used to identify various saltwater breeders.

The salinity tolerance and mosquito faunal succession and population dynamics in the breeding sites following monthly tidal flooding and seasonal rainfall will be analysed, as well as the dispersal potential of adult mosquitoes from saltwater breeding sites into inland areas.

Anthropophilic Sandflies in Machakos District

M.J. Mutinga

At Kibauni location in the Machakos District, studies were conducted using man as bait for sandflies both inside homes and at outdoor resting sites. Two species emerged as the major anthropophilic sandflies, *P. martini* and *P. (s) garnhami*, *P. (s) antennatus*, *P (s) bedfordi*, and *P. (s) schwetzi* were very occasional man-biters but were so few that they were not considered of any significance in the transmission of the disease. Table 4 below outlines the numbers manbaited by two workers at a termite hill site.

Table 4: Man-baited catches of *P. martini* and *P. (s) garnhami* From 7 to 8 p.m. at a Termite Hill at Kibaimi for ten days per month

Month	*P. martini*	*P. (s) garnhami*
March	50	10
April	79	11
May	29	9

P. garnhami does not persist throughout the year but *P. martini* does. Since previous studies have shown that *P. garnhami* can pick up experimentally infection of *L. donovani* when fed on infected volunteers, the feeding habits of this species strongly suggest that it is one of the vectors of Kala-azar in Machakos District in addition to *P. martini*. An isolation of leishmania organisms was made from *P. martini* and has been so far serologically typed as B2 serotype, a serotype to which *L. donovani* belongs. Investigations using isoenzymes is pending. Man baited feeding behaviour studies in nature indicate that *P. martini* is the major vector with *P. (s) garnhami* a secondary vector.

The impression smears and the cultures of the five isolates were without demonstrable parasites, an indication that the isolates were uninfective to hamsters and outbred albino mice by direct inoculation of promastigotes from culture media. The parasites which were inoculated directly from Kala-azar patient into hamsters, however, developed demonstrable parasitaemia in cultures and impression smears. It would appear from these observations that parasites inoculated directly from host to host are more readily infective than promastigotes in cultures.

Infectivity of Leishmania to Laboratory Animals

J.B. Kaddu and M.J. Mutinga

In order to investigate the capability of various species of sandflies to transmit leishmanial parasites under laboratory conditions it is ideal to let flies feed on infected animals. Because all leishmanial isolates are not readily infective to laboratory animals it is valuable to test the infectivity of various isolates to suitable laboratory animals. Studies were therefore undertaken in search of isolates which were capable of infecting laboratory animals.

Five leishmanial isolates (two from human and three from sandflies) were cultured in NN culture medium and then in RPMI-1640 culture medium. medium. The promastigotes were separated from the medium and then inoculated (intraperitoneally and intrasubcutaneously) in male hamsters and male outbred albino mice, so that each animal received over 2×10^6 parasites. One human isolate was made through direct inoculation of positive Kala-azar patient splenic aspirate. Some of the hamsters were treated with cyclophosphamide (an immunosuppresant) at doses ranging from 75 to 150 mg/kg (intraperitoneally) with the aim of suppressing the immunoresponses. The animals were maintained on unlimited supplies of pelleted diet, vegetables and tap water. They were sacrificed at various periods, post-inoculation, ranging from 24 to 225 days. Impression smears and cultures of tissues from the following organs were made: spleen, kidney, sternum, liver and heart.

Animal Model for Feeding Wild caught Phlebotomine Sandflies

M.J. Mutinga, D.J. Kaddu and L.W. Irungu

Wild caught sandflies were captured from rock crevices and termite hills and released into feeding

Table 5: Trial Feeding of Cooled Wild caught Phlebotomine Sandflies from Rock Crevices on Various Animals

HOST	*P. MARTINI*	*P.(S) BEDFORDI*	OTHER SPECIES	TOTAL	
	Unfed	Fed	Unfed	Unfed	Fed	Unfed
Dog	0	0	16	3		19
Rabbit	0	0	24			24
Hamster	0	0	42	1		43
Mouse	0	1	17		1	17
Guinea pig	1	0	3	1		5
Rat	0	1	9	1	1	10
Lizards	0	2	3		2	3
Total	1	4	114	6	4	121

Table 6 Trial Feeding of Cooled Human-Baited Phlebotomine Sandflies from Termite Hills

HOST	*P. MARTINI*		*P. S. BEDFORDI*		OTHER		TOTAL	
	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed
Rabbit	3			2			3	2
Hamster	10	11	1	19	1		15	30
Mouse	1	1	–	1	–	–	1	2
Guinea Pig	1	1	–	–	–	–	4	1
Rat	1	1		–	–		4	1
Total	13	14	1	22	1	1	18	36

cages containing various kinds of animals to find suitable hosts for feeding flies for experimental as well as for breeding purposes.

The flies were cooled prior to feeding. Flies collected from rock crevices which comprised mainly the reptilian feeding species had lower rates of feeding on laboratory animals than did those man-baited from termite hills (see Tables).

Results indicate that mammalian hosts (hamster, rabbit, guinea pig, mouse and rat) can be utilized for feeding anthropophilic sandflies.

Vector Parasite Relationship of Leishmania in Kenyan Phlebotomine Sandflies

J.B. Kaddu and M.J. Mutinga

Leishmaniasis, a zoonosis transmitted through the bite of female sandflies, is an important drawback to rural development in many parts of tropical and subtropical countries. Because of lack of a common life cycle for all species of the causative organisms one needs to have information regarding the vector-parasite interaction in the various vectors species, for the purpose of disease control. This kind of information is unavailable for Kenyan Phlebotomine sandflies. While arrangements are being made to establish a closed colony of sandflies in Kenya, investigations were started on various species of naturally-infected sandflies. This report concerns ultrastructural findings of *Leishmania aethiopica* in *Phlebotomus pedifer*.

Sandflies were captured from natural resting sites using standard techniques of suction tube. They were processed through standard techniques, dissected and their guts examined under a light microscope for presence of leishmanial parasites.

A small incision was made at the posterior part of the abdomen and the gut was carefully exposed and examined without a coverslip. When parasites were found, the gut was fixed with 2.5% glutaraldehyde in sodium cacodylate buffer and processed using standard techniques for electron microscopy.

In the thoracic midgut promastigotes were found in the alimentary canal. Flagella of promastigotes were observed embedded both amongst long microvilli and deep in the cytoplasm of the gut cells. Some of the tunnels in which the flagella were inserted were lined with membranous material. The flagella were attached on the walls of the tunnels by desmosomes (Fig. 7).

Fig 7 Transmission electron micrograph of the thoracic midgut showing flagella (F) of promastigotes inserted both amongst microvilli (MV) and in the cytoplasm of the gut cells (GC) Magnification: 17500 X

The factors influencing penetration of the flagella into gut cells are not well understood. Whether promastigotes preferentially invade a particular physiological and/or morphological form of cells has yet to be investigated.

Establishment of a Leishmania Bank

J.B. Kaddu and M.J. Mutinga

To be able to investigate vector-parasite relationships, infection rates and identity of isolated parasites, it is necessary to have access to a reliable source of parasites. A method of maintaining iso-

lates of leishmanial parasites has been established. Of the various methods, namely maintenance by passage in animals, culture, and by cryopreservation, the last is the cheapest and most reliable one. Its advantages are: it is cheap, it avoids the risk of loss of parasites through refractory animals, missed transfer and unexpected death of animals, it facilitates the maintenance of many strains, each in its original character, and it makes it possible to transfer the parasites without hosts thus eliminating feeding in transit and the carrying of undesired infections from organisms.

All new isolates of leishmanial parasites have been cryopreserved in liquid nitrogen. The old isolates previously maintained in solid carbondioxide have been transferred to the liquid nitrogen bank because the viability of parasites in liquid nitrogen (−169°C) is superior to that in solid carbondioxide due to the more effective arrest of biological activity. The viability of the transferred isolate has yet to be confirmed. Altogether the new and old isolates now number over 100.

One of the main problems in the isolation of leishmanial parasites under field conditions is contamination due to bacteria and/or fungus. The former is minimized by the standard use of antibiotics. Fungal contamination is more difficult to eliminate, however it was possible to separate leishmanial promastigotes form fungi in culture by chromatography.

Investigations into Animal Reservoir Studies

M.J. Mutinga and J.B. Kaddu

Investigations on animal reservoirs have been carried out at Kibauni Location in Machakos District. Emphasis has been made mainly on dogs, lizards and carnivores. A few animals other than those mentioned above have been included if accidentally trapped. Three Leishmania isolates have been made from lizard of family Gekkonidae. These are the first wild animal leishmania isolates from Machakos, a district which has been plagued with kalazar epidemic. The other leishmania isolates made from the location were from domestic dogs which have been typed to *L. donovani*, thus indicating that the dog is a domestic reservoir of the disease in Machakos District. The table below summarizes the work on the animal reservoirs. The examination of the tissues or slide smears have so far revealed blood parasites resembling Bebesia, from two genera.

Investigations into General Taxonomy of Immature Stages of Sergentomyia Species of Sandflies

M.J. Mutinga and L.W. Irungu

Various species of sandflies have been collected and fed on laboratory hosts to lay eggs. These were then placed in rearing cages for incubation and rearing to larval and pupal forms for comparative studies at both light and electrone microscopic (scanning) levels. Sandfly eggs from 5 species, *P. (s) bedfordi*, *P. (s) zanthomi*, *P. (s) antennatus*, *P. (s) lewisi* and *P. (s) affinis* have been obtained and are under investigation. Recent studies of the New World immature stages of phlebotomine sandflies indicate that species differentiation at the immature stage level is possible. Studies of this nature have not been carried out in the African continent on the group of the sandflies under investigation and consequently this will provide a new tool for sandfly identification at the egg and larval stages of the sandfly.

Development of an Efficient Sticky Sandfly Trap

M.J. Mutinga

The use of suction traps proved unsuccesful for trapping sandflies inside homes during the dry and the rainy seasons. A new trap was developed which proved to be very efficient. The new trap is suitable for studies of resting sites and sandfly population dynamics. It is made of plastic sheeting of 0.25 mm thickness hung on an adjustable metal frame or on wooden poles. The trap is coated with castor oil and set out at night either inside or outside homes around the termite hills. The trapped flies are collected in the morning. By means of these traps it will be possible to investigate infection rates and sandfly behaviour.

Hundreds of sandflies were captured inside houses by the traps (Table 8) whereas not a single sandfly was captured by suction tubes during the same period.

Table 7 Animals Captured in Kibauni Location, Machakos for Leishmaniasis Investigations by NNI's Culture and Tissue Examinations

ANIMAL	NUMBER EXAMINED	NUMBER POSITIVE	NATURE OF PARASITE
Domestic Dog	561	–	–
Genet Cats	23	2	Bebesia spp.
Mongoose	14	–	–
Civet Cat	1	–	–
Rock Hyrax	2	–	–
Ground Squirrel	1	–	–
Lizards	206	3	Leishmania spp.

Table 8: Sandflies Captured using Sticky-Sandfly Trap Inside Homes and Termite Hills

Month	No. Sandflies from two Homes	Sandflies from two termites Hills
August	646	257
September	171	174
October	434	214
November	486	377

Except for the month of September when the trapp g was done for 12 nights the rest of the months were covered in 20 trapping nights.

Studies on Taxonomic Characteristics of Phlebotomus Sandfly Vectors of Leishmaniasis

L.W. Irungu

Members of the *Synphlebotomus* complex, namely *P. martini*, *P. celiae* and *P. ransomeranae* are the vectors of visceral leishmaniasis in Kenya. The females of these three species are identical, hence the only clue to their identity, is the species of male predominating at the termitary. It is hoped that by carrying out studies on the immature and adult stages, it will be possible to separate the 3 species more accurately. This involves:

a) Rearing the flies
b) Studies of chorionic sculpturing of eggs
c) Chaetotaxy of 4th instar larvae
d) Studies on selected morphological parameters of the adult fly.

Rearing of flies

P. martini were caught by means of human bait at termite hills in Kibauni location. These catches took place between 6 pm and 10 pm every night. Flies were collected in glass vials when they landed to feed on man. They were cooled at −4°C for one minute and then released into feeding cages. The flies were fed on various animals, which had been shaven on the abdomen and anaesthetized with sagatal (sodium pentobarbitone at 26.4mg/kg body weight). The animals used included hamster, rabbit, mice, dog, guinea pig, rat and lizard. Engorged flies were placed individually into rearing cages lined with damp filter paper and provided with sugar until they laid eggs.

Eggs were counted and transferred into sterilised petri-dishes, covered with damp filter paper. They were kept in damp lunch boxes and placed in an incubator at 27°C and relative humidity ranging from 75–80%. The eggs were sprinkled lightly with liver powder or 'Ready' food on the 4th day. After hatching the larvae were provided with food daily and in order to prevent fungal growth the petridishes were cleaned daily. *P. martini* was reared only up to the 2nd instar larvae, after which the larvae died.

Studies on chorionic sculpturing of eggs

Some of the eggs laid by gravid females were processed for studies of the chorionic sculpturing using the S.E.M. Sandfly eggs were fixed in Carnoys fixative and then dehydrated through ascending grades of alcohol up to absolute alcohol. Debris was removed by using the ultrasonic

Table 9: A comparison of body measurements of *P. martini* based on flies collected in Kalawa (Machakos) and Marigat.

Parameters	(average of 20 flies) Kalawa	(average of 65 flies) Marigat
Body length (thorax + abdomen)	2.46 mm	2.3 mm
Head length (including dypens)	437	445
Head width	409	403
Labrum length	434	319
Antennal flagellomere III length	312	234
AIV + V length	.08	214
AIII/IV + V	0.98	1.1 mm
AIII/Labrum	0.64	0.74
Palpal seg 1 length	56	54
2	169	154
3	191	192
4	184	179
5	503	345
Palpal formula	1.2.4.3.5	1.2.4.3.5
Wing length	3.7	2.00
width	675	587
R_2 length	445	412
$R_2 + R_3$ length	330	295
$R_2/R_2 + R_3$	1.4 mm	1.4 mm
R_1 length	1.4 mm	1.1 mm
R_1/R_2	3.9 mm	3.0 mm

*Measurements in µm unless otherwise indicated

cleaner. Clean specimens were then double coated with carbon and gold in a vacuum evaporator and observed in a Jeol scanning electron microscope. Photographs were taken at magnifications between 200x and 3000x. Eggs of *P. martini* have shown interesting chorionic sculpturing. Further investigations will be carried out to follow the changes that take place to the chorionic sculpturing of eggs with time.

Chaetotaxy of 4th instar larvae

P. martini eggs have so far only been reared to 2nd instar larvae after which they died. Hence chaetotaxy of the 4th instar larvae has not yet been carried out.

Studies on adult morphology

It has been found that ecology attributes to the tendency of sandflies (especially those with a wide distribution) to form geographical races. Another influencing factor could be the delicacy of sandflies and their limited powers of flight. Currently *P. martini* from Kibauni, Machakos District and from Marigat in the Rift Valley are being studied. Later on, *P. martini* from various foci will be investigated. Table 9 gives some results obtained so far.

CHEMISTRY AND BIOCHEMISTRY RESEARCH UNIT

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Professor J.H. Law (1977)

Scientist in Residence

Professor F.J. Kézdy (1979)

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The Unit co-ordinated an International Scientific Working Group on 'The Use of Naturally Occurring Plant Products in Pest and Disease Control' from 12th – 15th May 1980. The principal papers and the discussion are being published as a book.

Introduction

D.L. Whitehead

The Unit is now capable of undertaking a wider spectrum of research especialy since Biochemistry acquired equipment for protein separation and purification. A capillary column has been borrowed to increase the resolution and capability of the Gas liquid chromatography system attached to the Mass Spectrometer. This will speed up analyses of pheromones now under study.

The Unit, still undertakes the services in support of the Programme as in former years.

Purification and characteristics of the major proteins in the eggs of the tick, termite, tsetsefly and shootfly

R.M.W. Vundla, D.L. Whitehead and F.J. Kézdy

Analysis of the vitellins provides an accurate biochemical method for assessing the action of gonadotrophic hormones. Also, the tick vitellin constitutes a potential antigen for inducing resistance against infestation in livestock.

With these goals in mind, a survey of the major proteins of tropical insect and acarine eggs was begun. New methods had to be devised to ensure conservation of the proteins in their natural state. Gel filtration in saline resulted in purification of the vitellin of *Rhipicephalus appendiculatus*, *Ornithodoros porcinus porcinus*, and *Macrotermes michaelseni*, as judged by their homogeneous behaviour during isoelectric focussing. In both species of fly examined, the large M.W. vitellin was conspicuously absent.

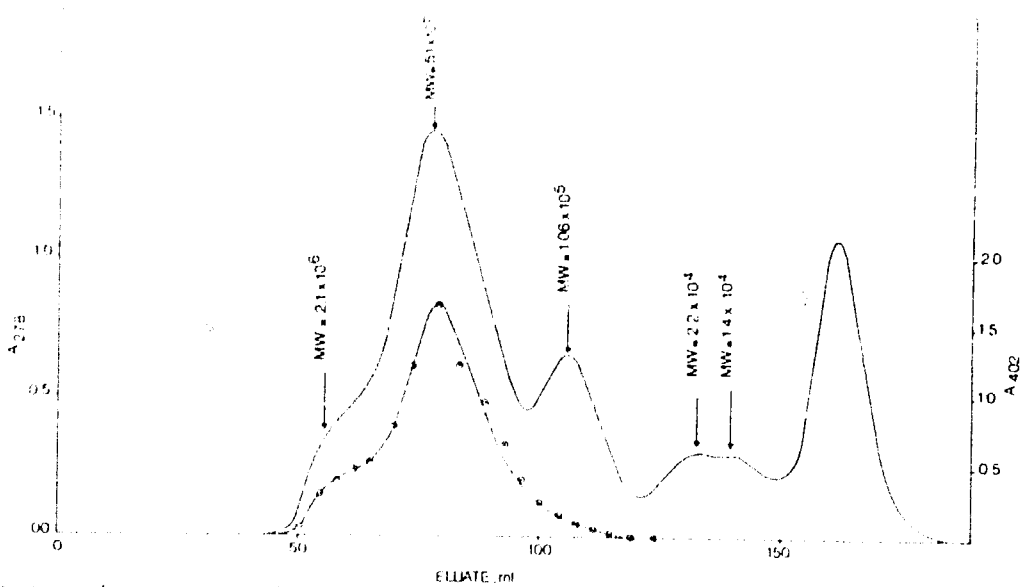

Fig. 1. Sepharose (CL-6B) gel permeation chromatography (1.6 x 80 cm column; flow rate = 40 ml hr⁻¹) of *Rhipicephalus appendiculatus* eggs homogenized in 0.02 M sodium phosphate buffer (pH 7.0) with 0.2 M sodium chloride.

The six major protein fractions in *R. appendiculatus* eggs have M.W's of 2.1×10^6, 5.1×10^5, 1.06×10^5, 2.2×10^4, 1.4×10^4 and $<1.2 \times 10^4$. (Figure 1). Haeme is associated with the two largest proteins only; the ratio of protein to haeme being identical. Both are composed of identical protein subunits of 9.7×10^4, 8.7×10^4 and 3.6×10^4 daltons (as determined by SDS PAGE).

The ratio of the two haemo-proteins is concentration dependent — the larger is a non-covalent tetramer of the smaller. In the presence of 8M urea the haeme dissociated quantitatively from the apoprotein, as shown by gel filtration using Sephadex CL-6B.

Isoelectric focusing showed that the vitellin is a single species with a pI of 6.5. It is present in the haemolymph of the mature female after feeding but not in the male. Therefore, it is incorporated into the egg without detectable modification.

Preliminary experiments suggest that the 1.06×10^5 dalton protein fraction, which is composed of at least three individual proteins (as seen after isoelectric focusing and ion exchange chromatography), is capable of binding ecdysterone but not ecdysone. Presence of these hormones in the egg was reported earlier. (More recently, GC with ECD is being used to determine the ecdysteroid concentration of egg extracts after silylation).

The ecdysterone binding protein in pure form could be used to produce antibodies in livestock which would sequester and inactivate the carrier proteins once ticks had engorged upon treated animals. Resistance to infestation by ticks conferred by immunization of cattle with whole tick extracts, described elsewhere in this report, might be due to just such a mechanism.

Chemical Identification of Termite Trail Pheromones

P. G. McDowell

Last year work was initiated into the identification of compounds which act as trail pheromones in *Macrotermes michaelseni*. Trail active extracts had been obtained by extraction of major workers at −16°C in hexane. Separation of the compounds has been undertaken and a number of compounds have been identified. A series of normal hydrocarbons of chain length C-23 to C-30 have been found together with several mono-methyl branched hydrocarbons of chain length C-25, C-26 and C-27. The two major components in these extracts have been identified as the straight chain alkenes 9-heptacosene and 9-nonacosene. Two dienes of chain length C-27 and C-29 are also present but the double bond positions have not yet been determined.

Trail bioassay has been a considerable problem and no single fraction or compound has yet been shown to be active. However, bioassay methods for this species are currently being reinvestigated and a comparison between workers and alates, both male and female, is being undertaken. Both male and female alate whole body extracts show gas chromatogram (GC) profiles which are almost identical to those of workers.

A second species is now under study, namely: *Trinervitermes bettonianus*. Bioassay for this species has been well established and trail active extracts have been obtained from whole body extracts of female alates, dissected sternal glands of female alates and from extracts of filter paper strips across which workers have been iduced to forage.

From all three types of extract a preparative GC fraction has been obtained, containing a common component, which displays high activity in the bioassay. From both dissected glands and whole body alate extracts, this fraction contains a single compound (greater than 95%) and activity is displayed even down to the picogram range. Chemical and spectroscopic data are currently being accumulated on this compound and so far the data suggest a diterpenoid structure. The small (< 5%) component which is present has been determined to be n-eicosane. (The major compound has a retention time slightly shorter than this compound). A second GC fraction, containing several components of higher M.W. than the principal active compound, displays trail activity. Hence there are secondary compounds which play a role in trail behaviour. These have not yet been investigated.

The monoterpene hydrocarbons mycene and limonene (predominantly the former) have also been detected in both worker extracts and alate extracts. These are not active in trail-following and their biological significance remains to be determined.

Juvenile Hormone Analysis

P. G. McDowell

The juvenile hormones are important insect hormones which are much used in the research carried out at the ICIPE. The commercially available hormones are very frequently impure, contaminated with several double bond isomers. Due to the labile epoxy group, degradation can occur in storage. For these reasons such commercial samples should be purified before use in biological experiments.

Gas chromatographic (GC) analysis of the available samples of JHI, II and III (on a 5% silar 5cp column, 3m long 2mm i.d. temperature 215°C isothermal) have shown levels of impurities ranging from 15 — 35%, which is unacceptably high. Consequently a routine purification of juvenile hormones has been established. The method of choice for purification of these sensitive compounds is high performance liquid chromatography (HPLC), since it can be carried out under mild

conditions unlikely to affect the sensitive epoxy-group. The following system was employed. An Altex Ultrasphere S column (5μ particles, 4.6mm i.d. length 230mm) was used with a solvent system consisting of hexane (containing water at 50% of the saturation level) with 3.5% diethyl ether. With this system JH III has been purified up to the 95% level and it is expected that JHI and II can be successfully purified with the same system.

Analysis of juvenile hormones at physiological levels in insects is an extremely difficult problem. However, attempts are currently being made to establish a sensitive method by employing capillary GC with electron capture detection (ECD). The termite *Macrotermes michaelseni* is being used as a test insect since the juvenile hormones are thought to play an important role in caste determination in the larval stages of the insect. Preliminary work by GC with flame ionisation detection (FID) has qualitatively indicated the presence of juvenile hormones in several larval stages of *M. michaelseni*.

Antifeedants and Fungicides from *Clausena anisata* (Rutaceae)

T. Gebreyesus and A. Chapya

During the screening of plant extracts for antifeedants using African armyworm *(Spodoptera exempta)* larvae in the leaf disc bioassay, the petroleum ether extract of *Clausena anisata* (Rutaceae) was found to be active. The extract also showed fungicidal activity against *Cladosporium cucumerinum*

The active compounds were isolated and purified by column and thin layer chromatography. Two compounds had feeding deterrence activity at 100 and 500 parts per million. These compounds were characterized from their physical and spectral properties as the known coumarins imperatorin and xanthoxyletin respectively. The identification was confirmed by gas and thin layer chromatography comparisons with authentic samples kindly supplied by Drs. Dreyer and Mester. Another coumarin 3 (1, 1- dimethylallyl)-xanthyletin, isolated from the same plant did not show feeding deterrence activity.

Xanthoxyletin also shows antifungal activity against *Cladosperum cucumerinum*. Other minor components in the same extract show fungicidal activity. Their isolation and identification is in progress.

A compound active against *C. cucumerinum* is present in methanolic extracts of bulbils and in the juice of mature plants. The fungicide is being identified so that it might be extracted industrially from sisal juice.

Shootfly oviposition-deterring pheromone

D.L. Whitehead, A.K. Raina, J.I. Jondiko, P.G. McDowell and F.J. Kézdy

When there is a choice available, presence of a chemical on a shootfly egg attached to the leaf of a sorghum seedling deters oviposition by other gravid *Atherigona soccata* females. (Raina A.K. J. Chem. Ecol., in press.)

Several methods of purifying this pheromone have been developed in order to identify it. This involved separation initially from substances derived from the leaf using high performance liquid chromatography (HPLC) and gel filtration. More recently a method of collecting eggs in the absence of the plant has been devised.

Pheromone activity was shown by bioassay to be present in certain fractions for which mass spectra have been obtained. Purer samples are now being obtained by solvent extraction of lyophilized washings from eggs laid on parafilm. The analogues of the deterrent are being prepared and tested prior to patent application.

Identification of Repellent Principles in Pyrethrum Extract

D.A. Otieno

It has long been known that the extract of pyrethrum displays potent repellent action against mosquitoes, biting flies, and other blood-sucking insects and acarina. However, the chemical identity of the ingredients of the extract which are responsible for this activity is not known. As part of the continuing investigation of plant constituents which influence insect development or behaviour systematic studies were started on pyrethrum extract in order to reveal the chemical identity of the compounds responsible for its repellent properties. These studies required (1) a separation procedure for isolating the ingredients of the extract in analytically pure form, and (2) an appropriate bioassay for evaluating the repellent effects. In fulfilment of the first requirement, a high performance liquid chromatographic (HPLC) procedure, which is suitable for isolating the natural pyrethrins and the many other components of pyrethrum extract has now been developed (ICIPE Annual Report, 1979). During the year under review, a series of bioassays have been carried out to determine the repellent effects of all the compounds obtained by HPLC from the extract. The bioassay for repellency used *Rhipicephalus appendiculatus* adults in a climbing test over 2 hr devised by Bar-Zeev.

In a typical test 20 adult ticks were released on to the platform supporting the climbing rods. The ticks were restricted to this platform by paraffin oil placed on the platform edges.

The test compounds were dissolved in acetone and soaked onto a piece of cotton fabric. The fabric was positioned 20cm above the base of the platform. The control fabric was soaked in acetone. The number of ticks to cross the treated cloth within the time limit provided the basis for calculating the repellency of the chemical by the application of Abbott's correction formula as follows:

$$\frac{\text{Number of ticks crossing untreated cloth} - \text{Number of ticks crossing treated cloth}}{\text{Number of ticks crossing the untreated cloth}} \times 100 = \%\ \text{Repellency}$$

Table 1 below shows the results of these assays.

Table 1: Repellent Activity of the Components of Pyrethrum Extract Against *R. appendiculatus* Ticks

Chemical	Dose	% Repellency
Jasmolin I	0.1 µg mm^2	47.1 ± 1.0
Pyrethrum I	0.1 " "	98.3 ± 0.3
Cinerin I	0.1 " "	100.0 ± 0.0
Jasmolin II	0.1 " "	93.6 ± 2.3
Cinerin II	0.1 " "	44.1 ± 4.3
Pyrethrosin	5.0 " "	81.6 ± 13.1
Taraxasterol	0.1 " "	35.3 ± 0.5

The ticks which crossed either the treated or the untreated cloth remained above the cloth, either at the top of the rod or somewhere between the top and the cloth. In control tests using untreated cloth on both rods, the majority of the ticks crossed the cloth during the test period. However, in the 2hr test of the cloth, treated with effective compound, most of the ticks approached the cloth but turned back at some distance from the barrier. Some ticks proceeded on to the treated surface(or at some point near it) became affected by the chemical. With more ineffective compounds, some ticks were able to cross the treated substrate. Therefore, both repellent and miticidal effects, due to contact with the chemical or its vapour were noticed.

As shown in Table 1, Cinerin I provided 100% protection as no tick crossed the treated cloth. At the time of writing this report, evaluation of the pyrethrin II ester was incomplete, and further tests were in progress to establish the dose response curves for Cinerin I, and other effective compounds and to assess the extent of protection against the ticks which is offered by Oleoresin sludge, green oils and pale pyrethrum extract. The repellent principles in Pyrethrum extract have, however, been identified as Jasmolin I, Pyrethrin I, Cinerin I, Jasmolin II Cinerin II, Pyrethrosin and taraxasterol.

The effect of trypanosomes *(T. brucei brucei)* on the activation of trypsinogen-like zymogens secreted by the midgut of *Glossina morsitans morsitans.*

R.W. Vundla, L.H. Otieno, D.L. Whitehead and F.J. Kézdy

By quantitating the amount of proteolytic enzymes in the teneral fly midgut, we observed that the presence of parasites in the blood meal from an infected Wistar rat delays the accumulation of trypsin-like enzymes (ICIPE Annual Report 1978). However, the quantity of enzyme present at the time of maximum proteolysis (24 hr) is unaffected. These observations pointed to the delay of activation of the enzymatic precursor. 1979). However, the quantity of enzyme present at the time of maximum proteolysis (24 hr) is unaffected. These observations pointed to the delay of activation of the enzymatic precursor. We have now demonstrated for the first time the presence of the precursor (zymogens). Their activation is delayed by the trypanosome.

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Ultrastructural and Histochemical Observations on Spermathecal Gland of *Glossina morsitans morsitans* Westwood

E.D. Kokwaro and J. Murnithi

It is generally considered normal for *Glossina* females to mate only once and sperms contained within a spermatophore are temporarily stored in the female uterus from where each individual sperm is activated and migrates through the opening in the spermatophore into the spermathecal duct and into the spermathecae. Within the spermathecae, sperms may last for the remainder of the reproductive cycle. Apparently very little is known about the nutritional factors sustaining the sperms in the spermathecae and the eventual release of the sperms from this region. Because of this gap in our knowledge, the work in progress aims at defining the structure of the epithelium lining the spermathecal receptacle to see whether it contributes to the maintenance and viability of the sperm, or whether the epithelium is merely a cuticular isolation chamber. Furthermore, the chemical composition of the spermathecal secretion is being studied.

As shown in Figure 1 the spermathecal receptacle consists of a monolayered epithelium with cuboidal cells. Transmission electron microscopy has shown that the cytoplasm of the receptacular epithelium contains the secretory cells with adjacent secretory vesicles, microvilli, mitochondria, ribosomes, rough endoplasmic reticulum and microtubules (Fig. 2). The presence of an extensive endoplasmic reticulum in the epithelial cells suggests that these cells are possibly synthesizing and secreting material into the receptacle that will be exported from the cell by the ductule and probably be incorporated in its secretion. It is possible that some of the material produced in the synthetic process is of a proteinaceous nature. The presence of a proteinaceous substance in the epithelium and lumen of the gland has been confirmed histochemically with the mercuric bromphenol blue technique. Another type of secretion produced by the same cells could be some type of carbohydrate. This may be the case because the secretory areas, ductule and secretions in the main lumen show a positive reaction with the periodic acid-Schiff (PAS) method, thus indicating the presence of carbohydrate substances. It is therefore likely that a mixture of carbohydrate and protein consti-

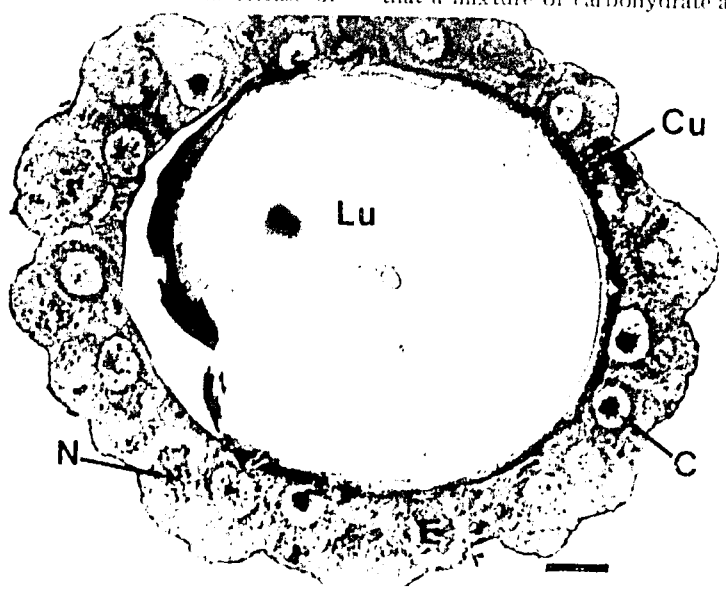


Fig 1: Photomicrograph of a mature spermatheca/receptacle. Epithelium (E), lumen (Lu), Nuclei (N), secretory cavities (C), cuticle (Cu). Bar = 6.2 Um.

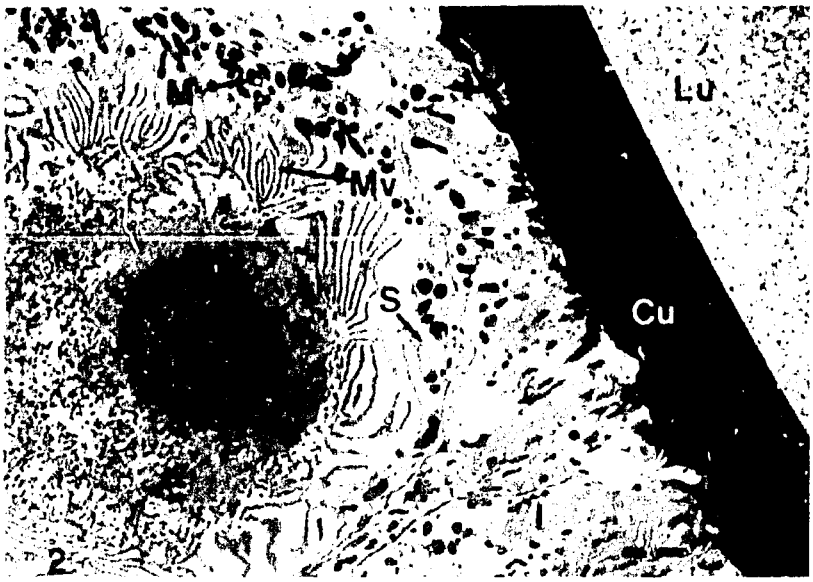


Fig 2 Survey transmission electron micrograph of the epithelial cell in the receptacle. Mitochondria (M), secretory cavity (C) with electron-dense material, microvilli (Mv), cytoplasmic infoldings (I), secretory vesicle (S). Bar = 1.8 Um

tute the spermathecal environment which sustains the sperm life

The Innervation of the Uterus in the Tsetse Fly *Glossina morsitans* Westwood

J.A. Kongoro

The uterus is important in tsetse reproduction being the organ in which fertilization of the egg from the ovary, gestation of the subsequent larval instars, and parturition of the third stage instar larva takes place. A nervous role has been implicated in the first and the last of the above events but more work needs to be done to clearly establish nervous involvement. Experimental work such as microsurgery, or injection of various pharmacological substances and indigenous nervous system extracts, and cytochemical studies on the substances released at nerve endings are some of the methods which could be used to elucidate nervous involvement in tsetse reproduction. As a background to such studies, the present work is aimed at giving detailed information on the innervation of the uterus, using light microscopic and ultrastructural techniques.

Using methylene blue, it was observed that the female reproductive organs were innervated by branches of the median abdominal nerve (MAN). Secondary branches (a, b and c) arising from these gave off nerves to the various parts of the reproductive system. Branch (a) gave off nerve a_1 which ramified the ventral side of the ovaries (Ov), the lateral and common oviducts (Ovid), nerve a_2 to the ventral side of the uterus (Ut), nerves a_3 and a_4 to the lateral side of the uterus while a_5 and a_6 innervated the posterior muscles of the uterus. Branch b gave off nerve b_1 to the anterior dorsal part of the uterus, b_2 to ramify the milk gland

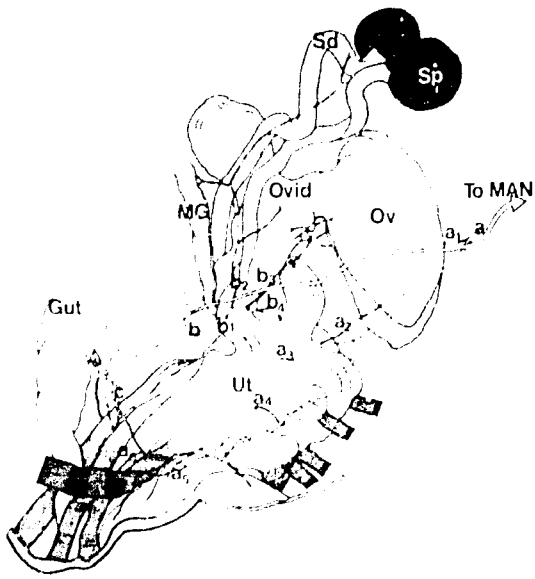


Fig 3: Innervation of the uterus in a three-day old *G. morsitans* female fly. Approximately X 175

(MG), the spermathecal ducts (Sd) in close proximity to the spermathecae (Sp) and the common oviduct, b_3 to ramify the dorsal part of the oviducts and the ovaries and b_4 to the anterior lateral part of the uterus. Branch c innervated the posterior part of the uterus.

Fig. 4 An electron micrograph showing a nerve in the dorsal part of the uterine wall. One of the axons (upper right) contains neurosecretory material N. X 15,750

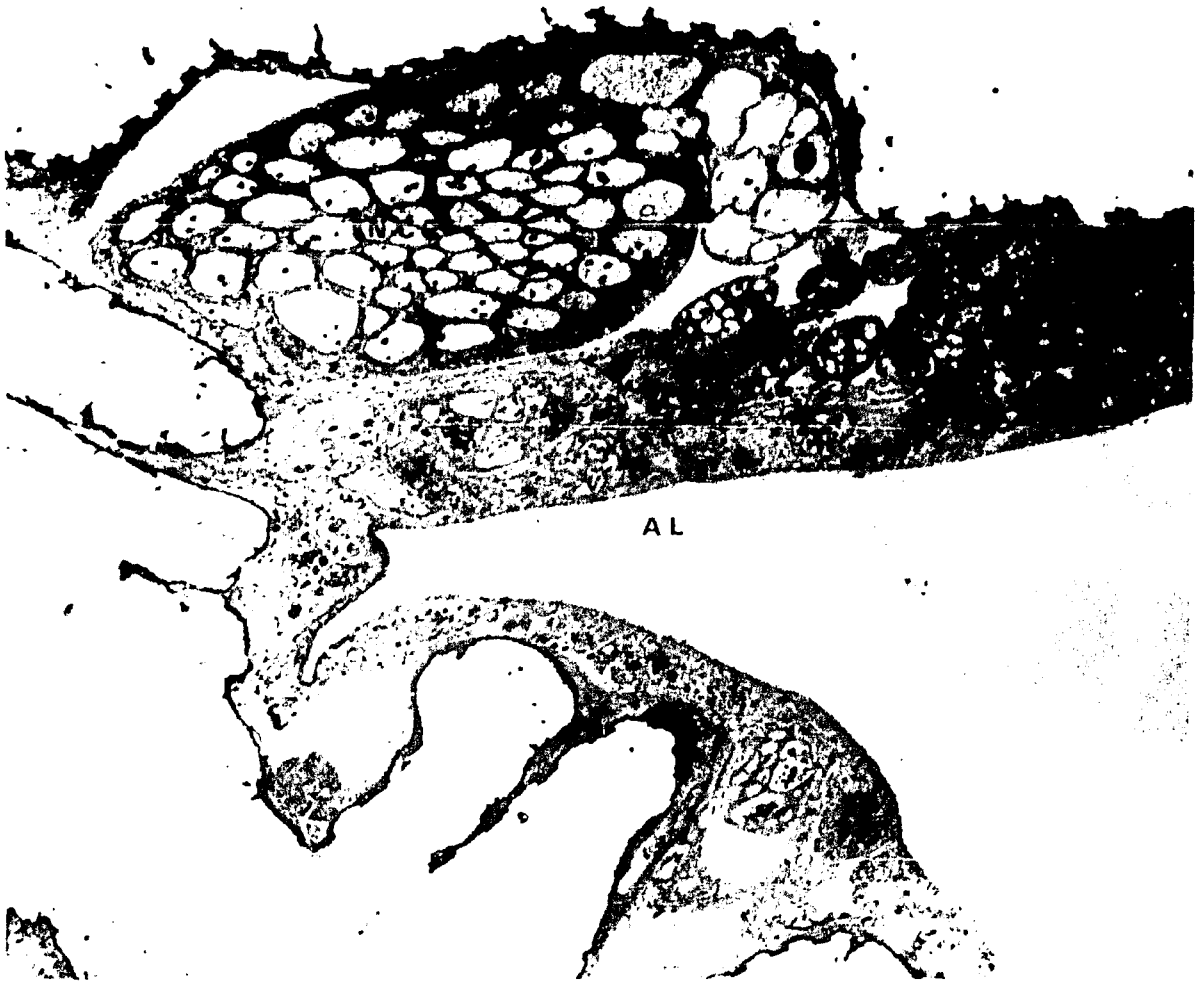
Ultrastructural studies showed the presence, in the uterine wall, of axons (A), some of which contained neurosecretory material (N; Fig. 4). This observation strengthens the case for the suggestion of a neurosecretory role in tsetse reproduction.

The Aorta as a Neurohaemal Organ in *Glossina morsitans*

L.R.S. Awiti

In most insects, the corpus cardiacum has been recognized as the main neurohaemal organ, from which the neurosecretory material originating in the brain enters the general circulation for transport to the target organs. Recent ultrastructural studies indicate, however, that in a number of insects, the wall of the aorta plays a major role in the release of neurosecretory material into the haemolymph. In the present study, the role of the aorta as a synthetic or release site for neurosecretory material in *Glossina morsitans*, has been examined using ultrastructural methods.

The aorta forms the anterior part of the tubular heart. The tube of the aorta has an irregular



[illegible] Low power electron micrograph showing the aortal wall [illegible] carrying neurosecretory material (NSM) after having [illegible] NCC [illegible]

thickness. Where the Nervus Corporis Cardiacum (NCC) from the brain enters the aorta, the wall becomes thick laterally and dorsally, and thin ventrally (Fig. 5). The inner and outer surfaces of the aorta are covered by connective tissue (stroma), about 1000Å thick. Both the aorta and the NCC are enclosed on the outside by another connective tissue.

The aortal wall is taken up mainly by the axons, mesaxons and glial tissue branching off from the NCC. The axons are fully packed with neurosecretory material (Figs. 6, 7). Between the darkly stained neurosecretory material in the axons are mitochondria (M) and neurotubules (arrow).

The axons close to the stroma, on either the aortal lumen (AL) side or haemolymph (H) side, have indistinct axolemma, and presumably the neurosecretory material leaves the axons at such points in the axolemma. The stroma itself, which is largely amorphous, has electron dense granules at intervals along it. These are, probably, the sites of release of the neurosecretory material into the haemolymph.

The pericardial cells on the wall of the aorta have scanty cytoplasm drawn out into thin strands close to the stroma or between the axons. The nucleus is large, compared to the rest of the cytoplasm. The cytoplasm does not contain any neurosecretory material. The present ultrastructural study indicates that in *G. morsitans* the aorta is a major neurohaemal organ, which does not itself secrete any intrinsic neurosecretory material.

Fig 6: [illegible] on the inner and outer surfaces by a stroma ST. Note the [illegible] and the most [illegible] myofilaments MF; on the wall NSM [illegible] AL [illegible] H [illegible] neurotubules arrows. X 15,250

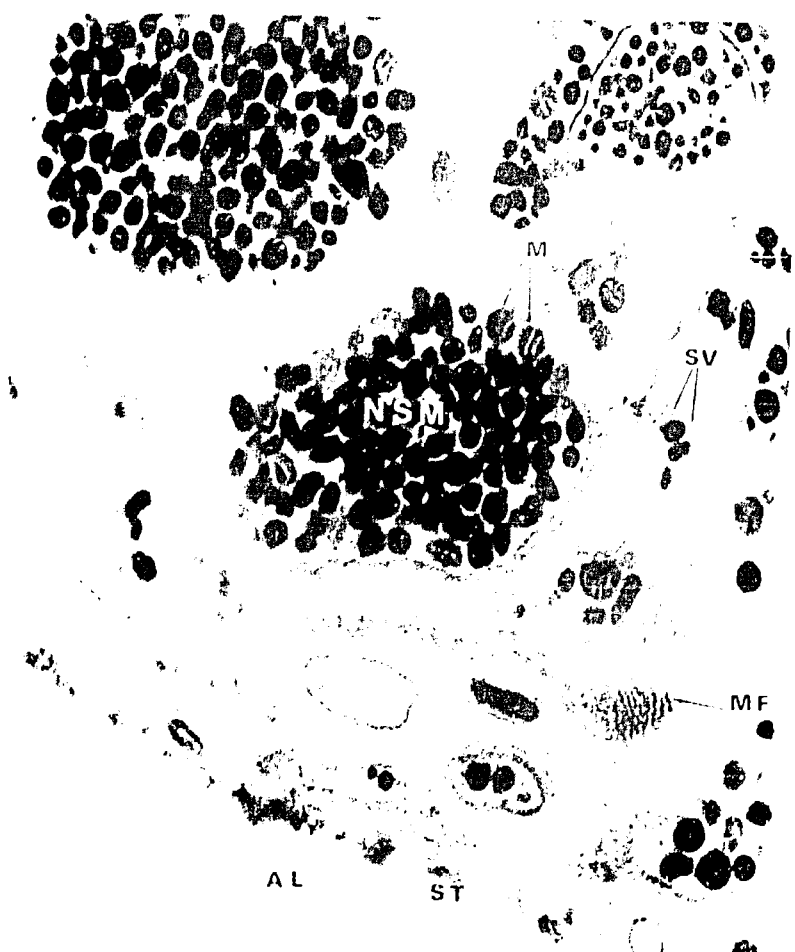


Fig 7: An electron micrograph showing the aortal wall close to the aortal lumen, AL. Note the thin axolemma around the axons (arrows) and the synaptic vesicles SV at the axonal endings. M, mitochondria; ST, stroma; MF, myofilament. X 45,000

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Electrophysiological Response from Tick Olfactory Sensilla Obtained by the Cut Tip Recording Method

S.M. Waladde

There is abundant information on the ultrastructural details of tick olfactory sensilla but there is almost no data on the response specificity of those sensilla. The objective of the continuing studies on two Ixodid tick species, *Rhipicephalus appendiculatus* and *Amblyomma variegatum* is to obtain some qualitative information about the "reaction spectrum" of the sensory cells innervating two of the dorsal surface sensilla apl and md3 on tarsus one (Fig. 1). The cut-tip recording technique has been adopted and perfected for recording electrophysiological response from tick sensilla.

Ultrastructural studies done on other tick species have shown that sensilla apl and md3 are multiply innervated and have several features in common with insect olfactory sensilla. Furthermore, the tungsten microelectrode technique was used to record electrophysiological responses from sensillum apl of the cattle tick *Boophilus microplus*. This technique has certain limitation in that the tip of recording microelectrode is superficially inserted into the base of the sensillum. Therefore the microelectrode mainly picks up signals from the cell bodies in the immediate vicinity of the recording tip. This method is quite inadequate

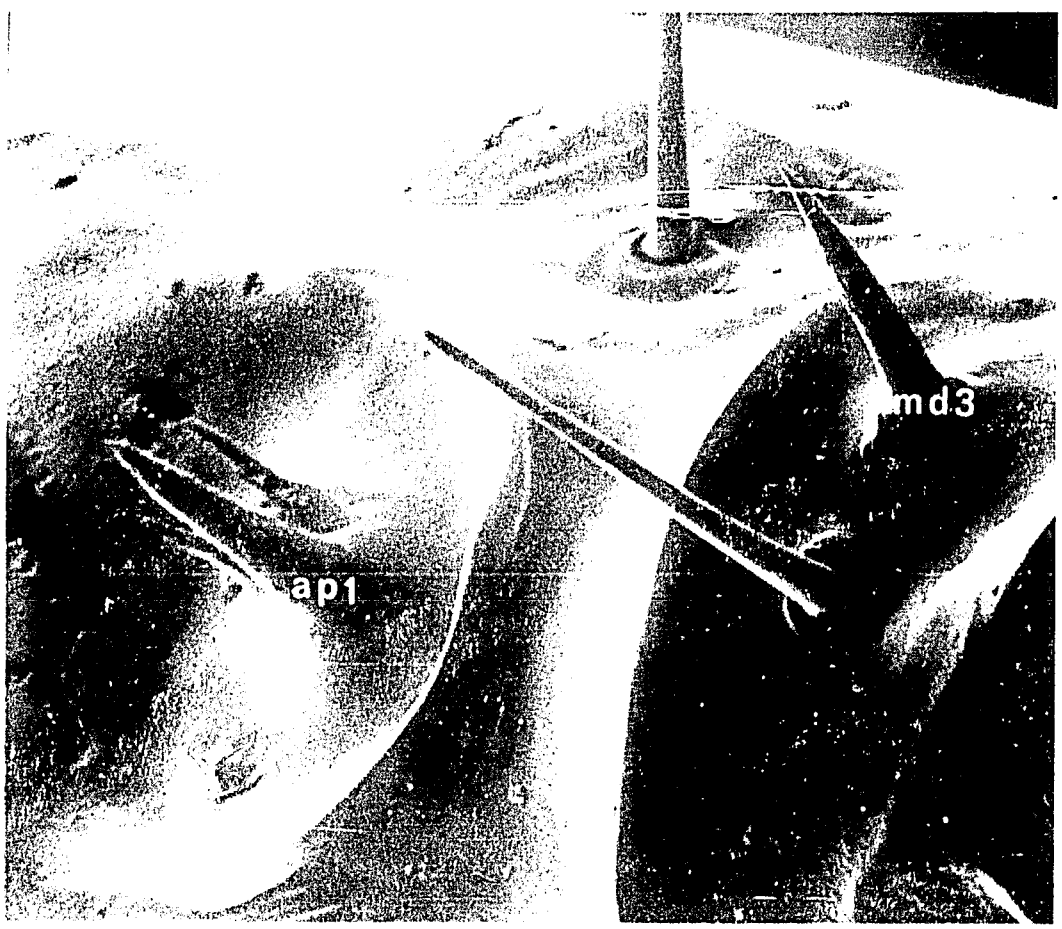

Fig 1 Scanning electron micrograph showing some of the dorsal sensilla on tarsus one of *Rhipicephalus appendiculatus* note the relative gap between apl and md3.

especially when one is recording from relatively large and multiply innervated sensilla. Another disadvantage is that the quality of the signals from the sensillum deteriorates fairly rapidly, this is possibly due to the damage caused by the recording electrode inserted into the base of the sensillum. Because of the above problems the cut-tip recording method originally used on the antennal sensilla of the silk moth *Bombyx mori* was adopted and modified slightly to suit the relatively short tick sensilla. This method is superior to the tungsten microelectrode method because it is possible to detect response patterns of several neurons within a single sensillum and one can easily record from more than one sensillum on the same preparation. Another advantage is that the preparation can be kept alive and functional for at least 24 hours and the quality of the action potentials from the neurons is quite consistent during that period.

The odour compounds tested in this work are selected on the basis of their shape, flexibility rotational characteristics and position of the functional groups. Other items tested include compounds with a known biological activity like 2–6 dichlorophenol as well as material of host and tick origins.

The available electrophysiological data gathered so far shows that sensillum apl and md3 are multiply innervated as shown by the different impulse amplitudes. It is apparent that the sensitivity of the neurons in those sensilla are different. For instance 2–6 – dicholorophenol stimulates certain neurons in each of the above s nsilla but on the whole neurons in apl are more sensitive to the above phenol than those in md3 (Figs. 2 & 3). Another difference is that the type of responsive neurons in md3 does not change with increases in the concentration of 2–6 – dichlorophenol but the reverse is true for sensillum apl. It remains to be established whether high concentrations of 2–6 – dichlorophenol similar to those tested in this work do really occur in nature.

By using a range of other compounds it has been observed that their effectiveness is a function of their molecular types and position of functional groups. Similar to observations made on the olfactory sensilla of other insects, the cell types in the tick olfactory sensilla will be identified by their "reaction spectrum" to those compounds which either cause excitatory or inhibitory responses.

Electrophysiological tests provide information about how a sensory cell or group of sensory cells respond to a particular stimulus but tells us nothing on how the animal as a unit responds to

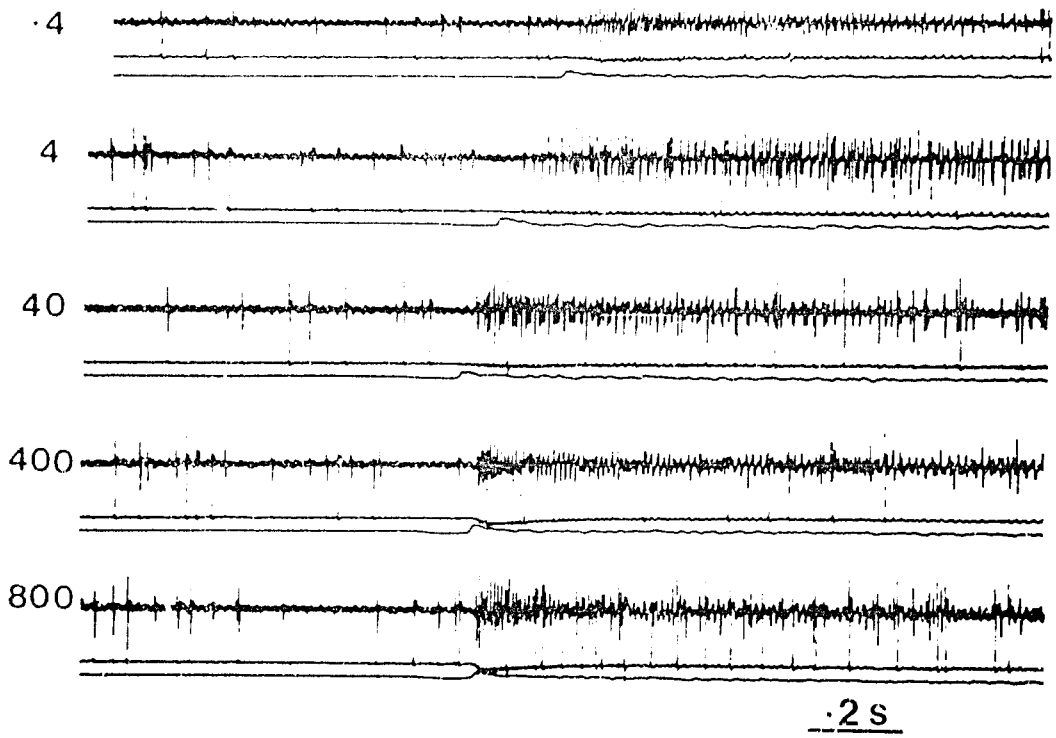

Fig 2: Electron physiological response from sensillum apl of *Ixodes variegatum*. Numbers alongside each trace represent manograms of 2–6 – dichlorophenol applied as a stimulus. The two lines below each trace indicate dc potential and stimulus marker respectively. A change in the baseline of the stimulus marker indicates the arrival of the stimulus coinciding with changes in the impulse pattern and frequency.

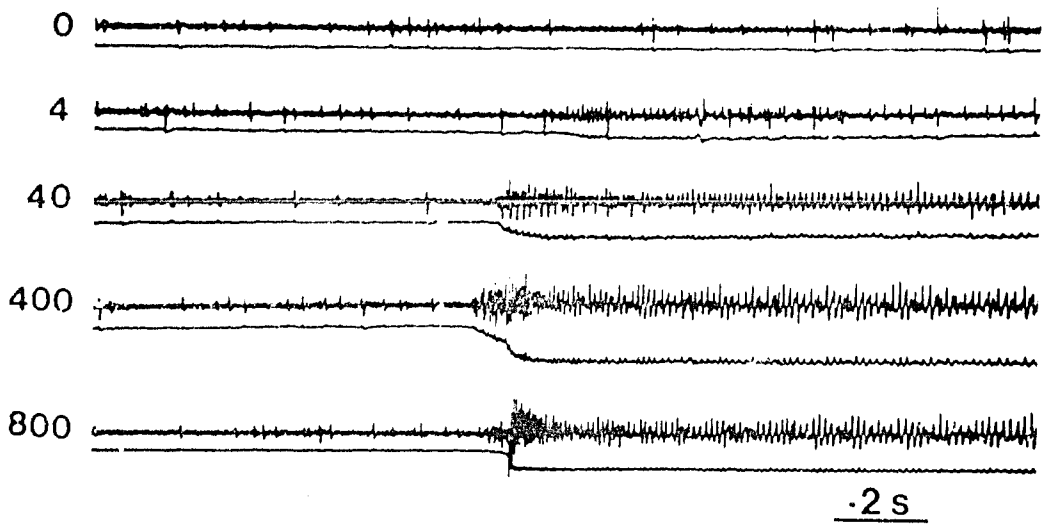

Fig 3: Effect of 2 6 dichlorophenol on the impulse pattern from sensillum mdA of *Amblyomma variegatum*. Numbers alongside each trace indicate nanograms of 2 6 dichlorophenol applied as a stimulus and each trace is accompanied by its decomposition trace.

that stimulus. The animal's response is a function of how the central nervous system integrates the sensory input and the resultant motor output. Controlled behavioural tests are thus conducted to provide the necessary information about the ticks behaviour towards known stimuli.

It is assumed that results from the current work will provide some information about the nature of adequate olfactory stimuli which influence tick behaviour. This kind of information prepares some of the prerequisites necessary for the evolution of useful biological control and other tick control or management methods.

Effects of Age and Hunger on The Pattern of Singing Activity in *Glossina morsitans morsitans*

R.K. Sami

It has been reported (ICIPE Annual Report, 1979) that both male and female tsetse flies showed a clear "U" shaped diurnal pattern of singing activity in LD 12:12. Those studies were restricted, however, to young flies and the first day after feeding. In the present investigation, the above studies were extended to determine the effects of age and hunger on the pattern of singing activity. Experiments were also conducted to determine whether or not singing is under endogenous control.

Teneral flies were fed and then placed individually in a plastic vial (4.5 x 3.0 cm) sealed with polythene gauze at both ends. The vials were then placed in the acoustic laboratory until the next day when these young flies (2 days old) were tested in a light: dark cycle (LD 12:12), in constant darkness (DD) or constant light (LL) over a four day period. Mature males and both mature virgin females and pregnant females (8 days after emergence and fed daily) were similarly prepared and tested in LD 12:12 for 5 days. When the flies were being tested, no bloodmeal was offered to them. Recordings of singing were taken by listening to each fly at hourly intervals between 0830 hours and 1730 hours each day.

In the case of young flies, as shown in Fig. 4, a clear diel pattern of spontaneous sound producing activity emerged in LD 12:12 on day 1. Both males and females showed a distinct "U" shaped pattern of singing activity during the photophase. The peak activity occurred during the early morning after which singing declined steadily till around midday when it reached the lowest level. In the late afternoon a second peak occurred at 1630 or 1730 hours. In total activity and duration, the afternoon peak was less than half of the morning peak. Singing during the scotophase was negligible. Singing activity on day 2 was characterised by a morning peak only. The afternoon peak was completely absent. The morning peak on the second day was considerably less than on the previous day. On day 3 and day 4 virtually no singing occurred and most flies died of starvation during these days. No significant difference between the pattern of singing in females and males (X^2 (36) = 22.07) was observed.

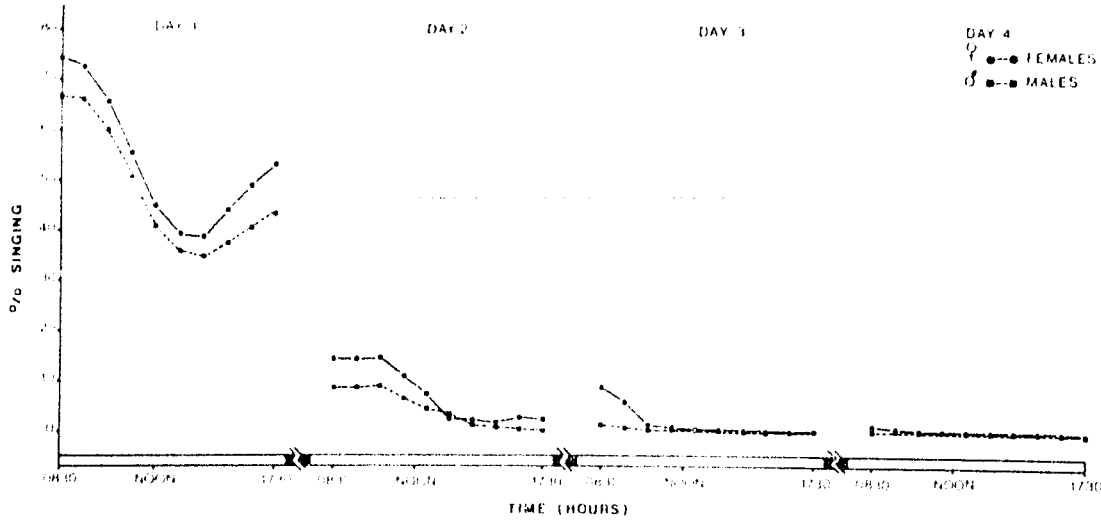

Fig 4: Sound production in young *Glossina morsitans morsitans* in LD 12 : 12, expressed as a mean percent of the flies singing (ordinate) against time of day (abscissa). Solid circles indicate mean percent of female flies singing and solid squares indicate mean percent of male flies singing. Dark bands along the abscissa show the scotophase period and the open bands indicate the photophase period. Three point sliding means were used to smoothen the curves.

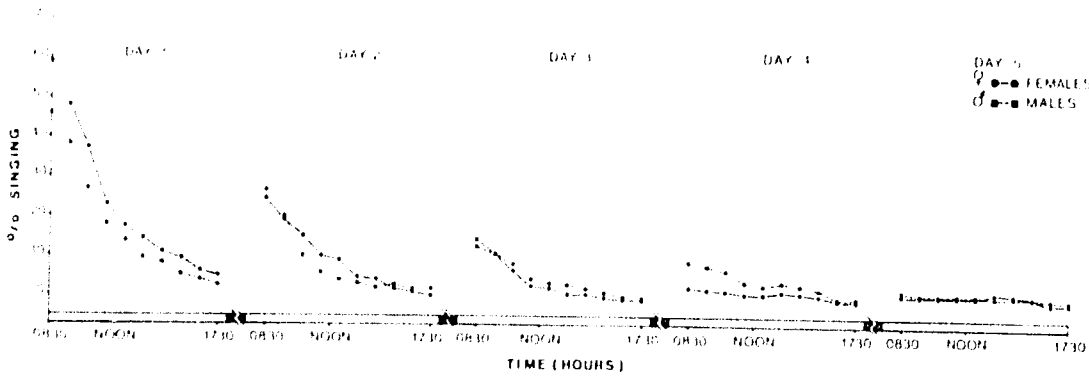

Fig 5: Sound production in mature *Glossina morsitans morsitans* in LD 12: 12. Solid circles indicates mean percent of mature virgin female flies singing and solid squares indicate the mean percent of male flies singing. Refer to Fig. 4 for other details.

Fig. 5 shows the pattern of sound production in mature male flies and mature virgin female flies in LD 12:12. Singing activity of both male and female flies was characterised by the complete absence of the afternoon peak on all days. Less than 10% of the experimental flies produced sound on day 4 and there was virtually no singing on day 5. Although females seemed to be singing more than males during the five day period, the difference was not significant (χ^2 (28) = 38.80).

The pattern of sound production in the case of mature pregnant females (Fig. 6) was not significantly different from that of mature virgin flies (χ^2 (36) = 47.31), although in the case of pregnant flies virtually no singing occurred from day 3 onwards.

In order to determine singing activity levels with advancing starvation, the mean percentage singing per day was calculated. It became quite clear that singing levels decrease with advancing starvation. In young flies there was a sharp fall in singing levels after day 1 of feeding (Fig. 7A). In the case of mature male flies, mature virgin females (Fig. 7B) and pregnant females (Fig. 7C), however, there was a more gradual decrease in singing with advancing starvation. Similar trends became evident when the flies were subjected to continous light or dark conditions (Figs. 7D and 7E respectively).

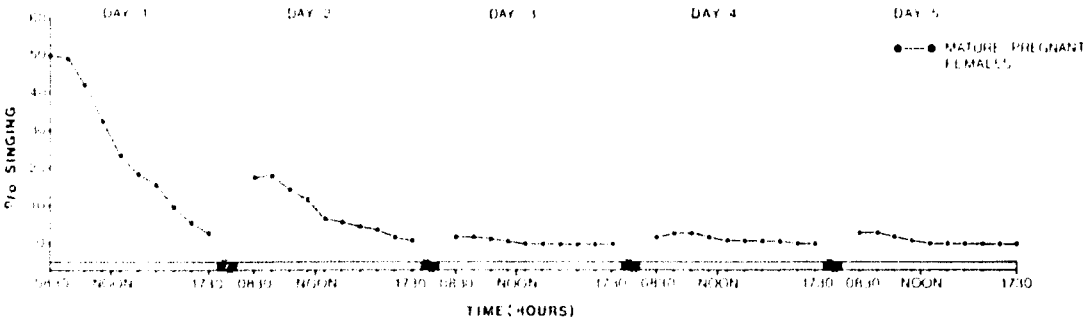

Fig 6: Sound production in mature pregnant female *Glossina morsitans morsitans* in LD 12: 12. Refer to Fig. 1 for other details.

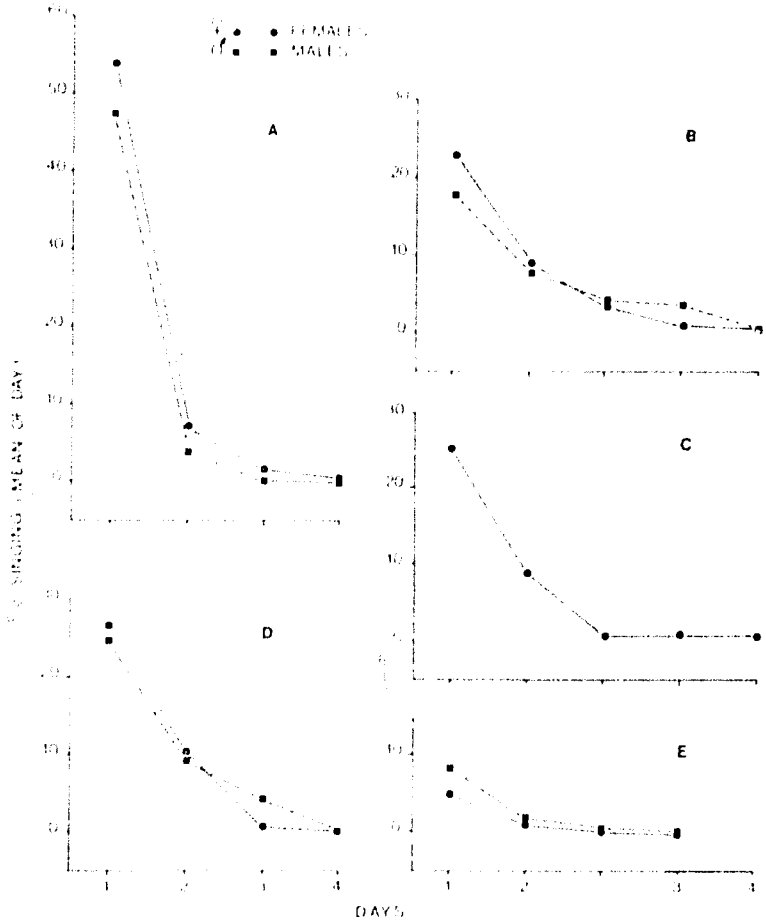

Fig 7: Singing activity levels with advancing starvation expressed as mean percentage singing per day. Solid circles indicate mean percentage singing per day in case females and solid squares indicate mean percentage singing per day in case of males.

(A) Singing activity levels in young flies (LD 12 : 12)

(B) Singing activity levels in mature males and mature virgin females (LD 12 : 12).

(C) Singing activity levels in mature pregnant females (LD 12 : 12)

(D) & (E) Singing activity levels of young flies in constant light (LL) and constant darkness (DD) respectively

Although in young flies the afternoon peak was evident on day 1 only and was virtually absent in mature flies of both sexes on all days, singing in LD 12 : 12 basically seems to follow the "M" shaped pattern typical of other diel responses by *G. morsitans* such as the rhythms of spontaneous flight activity, optokinetic responsiveness, olfactory responsiveness, probing responsiveness and field biting activity.

In contrast to other behavioural rhythms however experiments in continuous light (LL) and continuous darkness (DD) indicate little evidence of a clear circadian rhythm of sound production. Singing may therefore be a direct response to exogenous factors or may be a manifestation of some behavioural activity the meaning and significance of which needs to be investigated.

The successive decrease in total daily singing activity during the course of starvation is in sharp contrast to other behavioural patterns such as spontaneous flight activity, visual responsiveness and probing responsiveness where activity and responsiveness increase exponentially during the course of starvation.

The relationship between singing activity levels and the degree of starvation suggests that the average daily singing may be related to the nutritional state of a fly and that some feature of the metabolic reserves may ultimately control singing activity. That singing activity decreases with advancing starvation can be explained by the hypothesis that with increasing starvation, the fly saves all its energy for host finding activities and that singing has no immediate survival value to the individual at the particular moment. Moreover, the decrease in singing during the course of starvation in contrast to other investigated behavioural patterns implies that singing is not a by-product of other activities, but should be considered as an independent behavioural pattern.

Feeding Deterrent Receptors in the Last Instar African Armyworm *Spodoptera exempta* (Wlk.) (Lepidoptera: Noctuidae): A Study using Salicin and Caffein

J.V. Clark

Although the effect of antifeedants which produce disruption of receptor function have been studied in relation to the African armyworm, *Spodoptera exempta* (Wlk.), the presence of specific receptors sensitive to feeding inhibitors similar to those reported for a number of other lepidopterous larvae have not previously been demonstrated. For this reason a study has been undertaken using sixth instar *S. exempta* to determine the sensitivity of the larvae to two compounds, salicin and caffein, known to stimulate feeding inhibitor receptors in other lepidopterous larvae and also to determine the effectiveness of these compounds as feeding deterrents.

Electrophysiological evidence suggests that the only mouthpart receptors sensitive to the two test chemicals are the lateral and medial sensilla styloconica, and that the galea palpi, labial palpi and the labral sensilla coeloconica are not sensitive to salicin and caffein, although there is some inconsistent response from the labral sensilla to caffein. Many lepidopterous larvae possess feeding inhibitor receptors in their styloconic sensilla and similar receptors have also been reported on the labrum. It may be that the salt sensitive cells in the labrum of *S. exempta* respond to other feeding deterrents. Electrophysiological evidence suggests that it is the sodium chloride sensitive cells in the sensilla styloconica that respond to salicin and caffein. This is borne out by other evidence; in the lateral sensillum, two out of four neurons are sensitive to phagostimulants (sucrose and adenosine) and two to sodium chloride. It is likely that it is these latter two neurons that respond to salicin and caffein. In the medial sensillum one cell responds to sucrose and meso-inositol, and the remaining three respond to sodium chloride at various concentrations. Similarly it is likely that two of these three sodium chloride sensitive neurons are involved in the response to salicin of the medial sensillum.

Recording with mixtures of caffein and sucrose from the lateral styloconic sensillum did not show any disrupting effect on the lateral sucrose receptor by caffein, nor did prolonged contact (3 minutes) of the receptor with caffein solution (10^{-2}M) affect its subsequent response to a sucrose solution (10^{-2}M). The feeding tests conducted with salicin and caffein suggest that both compounds inhibit feeding, but not completely, at the concentration used. The maxillary cautery experiments suggest that this inhibition of feeding is still present in the absence of maxillary input, and further experiments suggest a sensitivity to salicin, caffein and sucrose in larvae with maxillary and labial palpi cautery and labrectomy. This fact is interpreted as indicating the presence of other chemoreceptors in the buccal cavity.

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Epizootiology of *Coelomomyces indicus* infections in the mosquito host, *Anopheles gambiae* larvae

W.A. Otieno, M.O. Odindo, D.M. Sabwa, S. Muti

Aquatic fungi of the genus *Coelomomyces* are parasites primarily of mosquito larvae. There are a few observations recording their presence in adult mosquitoes, but the effect of the fungus infection on the larvae is usually death before pupation. The possibility of using these fungi as agents for the biological control of mosquitoes deserves serious attention, and the conduct of meaningful experiment to evaluate any control potential the fungus may have should be enhanced by having information concerning the activities and responses of the parasite in its natural environmental setting. After careful searching, a site has been found that will permit the examination under field conditions of several aspects of the mosquito host fungus relationship. In mid 1979 an infection by *Coelomomyces indicus* of a population of the malaria vector *Anopheles gambiae* breeding in Iwamoni, Kenya Coast, was discovered. The level of infection was as high as 100% at times in the larvae collected at various site before the pool dried due to the setting in of the dry season. In 1980 a study was initiated to sample the pool throughout the breeding season of the host species. During this time data were gathered systematically on the level of fungal infection in the larval population.

Larvae of *Anopheles gambiae* were collected with a porcelain dipper from two shallow ponds, Mwamoni I and II, around Rabai, Kenya Coast. In taking two weekly samples of 100 dips each, the entire portion of each pool supporting larval growth was covered. It was determined that 100 dips produced an adequate sample of the larval and pathogen populations in which the percentage of infected larvae observed was not dependent on the number of larvae in the sample. Larvae were sought in the most likely resting or feeding places in the pool, e.g. in shade near emergent plant stems, along the bore line and moving the floating debris.

Larvae collected were brought immediately to the laboratory, where they were inspected microscopically for fungus infections not later than the

Table 1 Number of *Anopheles gambiae* larvae collected from Mwamoni Pool I, and the number and percentage infected by Coelomomyces indicus

Date of collection	larvae	Infect. larvae	% Infec. larvae
Aug. 12	—	—	—
Aug. 20	10	7	70%
Aug. 22	3	1	33.3%
Aug. 25	5	3	60%
Aug. 28	15	8	53.3%
Sept. 1	4	3	75%
Sept. 3	5	3	100%
Sept. 5	24	20	83.3%
Sept. 8	40	40	100%
Sept. 10	25	10	40%
Sept. 12	39	16	41%
October (Dry month)			
Nov. 10	0	0	0%
Nov. 14	11	8	72.7%
Nov. 17	0	0	0%
Nov. 20	39	21	53
Nov. 24	16	16	100%
Nov. 27	25	20	80%

Table 2 Number of *Anopheles gambiae* larvae collected from Mwamoni Pool II, and the number and percentage infected by *Coelomyces indicus*.

Date of collections	larvae	Infect. larvae	% Infected
Sept. 1	7	6	85.7%
Sept. 3	10	9	90%
Sept. 5	2	2	100%
October (Dry month)			
Nov. 10	0	0	0%
Nov. 14	8	8	100%
Nov. 17	0	0	0%
Nov. 20	14	11	78.6%
Nov. 24	15	13	86.7%
Nov. 27	1	1	100%

day after collection. The number of infected and uninfected larvae and the percentage of infected larvae for each sample were recorded (Table 1 and Table 2). Definite identification of the fourth instar larvae was made, and the lesser instars were determined to species as possible.

In attempts to obtain experimental infection of larvae of *Anopheles gambiae* by *Coelomomyces*, Muspratt (1961a) was unable to obtain repeated infections. He felt that, "given suitable climatic conditions", he could probably be successful. Since the percentage infection rates in nature by *Coelomomyces* have been reported to vary from one ecological area to the other, the environmental data presented in this preliminary report, although representing only a single season, may be an important step toward understanding the epizootiology of *Coelomomyces indicus* along the Kenya Coast.

A comparative percentage infection of the two pools indicates that the fungus, *Coelomomyces* reaches higher levels in Mwamoni Pool I than Pool II. This could probably be due to one or more factors of the physical environment operating differently in the two pools.

The observation that a higher percentage of infection occurs in fourth instar larvae than in the lesser instars may be interpreted to be a result simply of longer exposure of the fourth instars to the inoculum in the water. However the possibility exists that the infection seen in a fourth instar larva might have occurred initially in a lesser instar, and the fungus had not developed in the lesser instar to a detectable stage at the time of sampling.

Coelomomyces indicus: Its distribution and the ecology of infection pools along the Kenya Coast

W.A. Otieno, M.O. Odindo, D.M. Sabwa, S. Muti

Coelomomyces indicus a highly pathogenic fungus to the malaria transmitting mosquito, *Anopheles gambiae* was first recorded in June from temporary pools at Kongowea, Mombasa. In 1980 the knowledge on its geographical distribution has extended to two other areas, Mwamoni I and Mwamoni II both in Kenya Coast. After the discovery of *Coelomomyces indicus* from these site, investigations were initiated on the physical ecology of the infection pools. In the three localities where *Coelomomyces* was active, two pools were permanent infection sites, while the other host larvae were attacked only intermittently.

In an effort to describe the conditions surrounding infection of larvae, measurements of temperature, hydrogen ion concetration, pool size, bottom sedimentation, turbidity and biotic composition were made. pH was read on pHydrion paper (Micro Essential Laboratory); temperature readings were taken using a thermometer; and turbity using a white disc.

The main features of the observations can be summarized as:

(i) Temperature

The mean temperature for infected pools was 31.3°C for 8 readings, with a range from 26°C to 39°C.

(ii) Hydrogen ion concentration

Hydrogen ion concentration of 8 samples taken over a four month period at intervals of one week from the three infection pools gave a range from 6.00 to 8.00 (7.30 mean).

(iii) Pool size

All the infection pools varied from relatively small structures during the dry spell to large bodies of water (over 5,000 litres) in the rainy season

(iv) Bottom sedimentation

Two of the infection pools (Mwamoni I and II) have black clay soil; while the Kongowea site has loam soil.

(v) Turbidity

This varied from as low as 0.38" to as high as 1.00" (with a mean of 0.69") in 8 reading samples

(vi) Biotic of pools

Biotic composition depends on the stability of pools. In the more permanent parts of the pools the following organisms were sampled: large numbers of the orange copepods, *Tigriopsus* sp, Cladocerans, amphipods, ostracods, dysticids, hydrophilids, corixids and tadpoles.

Further investigations are now in progress to compile data on environmental parameters pertaining to situations in non-infected pools for comparative purposes.

Fine Structure Studies on the Sporangia of *Coelomomyces indicus*, a fungal pathogen of *Anopheles gambiae*

W.A. Otieno, E.D. Kokwaro, P. Lisamulla, P.A. Amutalla

Coelomomyces an obligately parasitic fungus occurring in the coelom of mosquito and a few other diptera larvae, was first described by Keilin in 1921. Couch (1945) recognized the blastocladiaceous nature of this genus and placed it in a new family, the Coelomomycetacea in the order Blastocladiales. More than 30 species of *Coelomomyces* have been described with all but 1 occurring in mosquito larvae. Most species are host specific, hence proper identification of the host mosquito is important. Species determination is also based on the size, shape, and surface structure of the resting sporangia; the type of sporangia present, whether thick or thin walled or both; and mycelial characteristics as revealed by light microscopy.

However, recent advances indicate that the Scanning electron microscope (SEM) has become a very powerful tool in providing additional taxonomic information. The present study is aimed at gaining an understanding of the surface structure of sporangia using SEM.

Sporangia for study were obtained from freshly collected mosquito larvae, from larvae preserved in 10% formalin or from larvae dried on filter paper. In all cases the infected larvae were dissected in distilled water so as to disperse and clean the sporangia. The sporangia were transferred directly to quartered 18 mm^2 coverslips that were attached to specimen stubs for the SEM or were collected on 0.45-micron (M) Millipore filter paper by using a Swinnex-13 filter unit. Thoroughly dried specimens were coated with carbon followed by a gold-palladium alloy. Preparations were examined with a Jeol Scanning electron microscope.

Fig 1 and Fig 2 show that the sporangial wall is football-shaped and arrayed with irregular anastomosing ridges which are separated by striae that are positioned perpendicular to the ridges. The demonstration here by SEM of striae between the ridges confirms the report by Couch (1945) that such striae are present in these species although not mentioned in the original description of Iyengar (1935).

Fig 1: A sporangium (S) of a fungus, *Coelomomyces indicus* X 400

FIG. 2 An SEM micrograph of the sporangial wall of a fungus, *Coelomomyces indicus* showing ridges (r) striae (s) X 2000

Laboratory Studies on two species of Cyclops, *Microcyclops minutus* and *Mesocyclops pilosus*

D.M. Sabwa, W. A. Otieno, M.O. Odindo, S. Muti

Studies were carried out on three factors that may affect the effectiveness of cyclops as intermediate hosts in induction of *Coelomomyces* epizootics in the field. These studies included the susceptibility of two cyclops species to *Coelomomyces indicus*, their salinity tolerance, and the survival of cyclops in a dried-up pond.

Susceptibility of different cyclops species

Two groups of cyclops from two different pools were brought into the laboratory and identified as *Microcyclops minutus* and *Mesocyclops pilosus*. The colonies were maintained separately in the laboratory on an egg yolk diet. *Coelomomyces indicus* resting sporangia were obtained from field collected larvae that had been stored at 4°C on moist filter paper for approximately two months. For each test, one fully infected larva from various sources was teased apart in a drop of distilled water in a plastic transparent container. After about 48 hours, 50 cyclops were added. One hundred second instar larvae of *Anopheles gambiae* were then added. Water temperature was usually 27 ± 1°C.

Infections occurred consistently in the containers with *Microcyclops minutus*, but not in those with *Mesocyclops pilosus*. Infections usually appeared around the 13th day.

The water source had no effect on the outcome of the experiments.

Salinity tolerance

Microcyclops minutus and *Mesocyclops pilosus* were exposed to various concentrations of sea water for two hours, after which the mortality percentage was recorded. The tests were carried out in four replicates at each concentration. Both species were found to tolerate salinities of up to 30% sea water, after which tolerance decreased sharply, reaching 100% mortality for *Mesocyclops pilosus* in 40% sea water.

Table 3: Susceptibility of two cyclop species to *Coelomomyces indicus*

Water source	INFECTIONS	
	Microcyclops minutus	*Mesocyclops pilosus*
Tap water	+	
Distilled water	+	-
*Pond I water	+	
Pond II water	+	-

*Pond infected with *Coelomomyces*

Table 4: Salt water tolerance for *Microcyclops minutus* and *Mesocyclops pilosus*

% sea water	% mortality	
	Microcyclops minutus	*Mesocyclops pilosus*
0	0	0
0.63	0	0
1.25	0	0
2.50	1.25	1.25
5.0	2.5	0
10.0	5.00	0
20.0	5.00	1.25
40.0	73.8	100.00
80.0	100.0	--
160.0	--	--

Survival of cyclops in dry soil

Soil was collected from a known cyclops habitat after a dry spell of about two weeks. In the laboratory, the soil was put in petri dishes and flooded with tap water. Observations were carried out for emergence of cylops, and for the developmental stage at the time of appearance. This was repeated after two days. Each set of experiments was carried out in three replicates. The cylops emerged from the soil in the copepodid stage, about 18 hours from the time of flooding.

The potential of *Coelomomyces* as a biological control agent depends to a large extent on the presence or successful establishment of a copepod (Cyclops) which have been demonstrated to serve as an intermediate host for the fungus, *Coelomomyces* species by several workers.

Whisler et al. (1974) reported the discovery of *Cyclops vernalis* as the intermediate host of *Coelomomyces psorophorae*, which attacks the larva of *Culiseta inornata* in North America. This announcement was of major significance and has had a direct bearing on *Coelomomyces* research currently being pursued in various laboratories. Similarly Pillai *et al.* (1976) demonstrated that *Tigriopus* sp. near *angulatus* served as the intermediate host for *Opifex fuscus* and *Aedes australis*.

Cyclops are found in most mosquito breeding sites, and seemingly even in dried up ponds. Thus successful exploitation of *Coelomomyces* as future biological control agent will necessitate identifying the right cyclop species (as an intermediate host) for each *Coelomomyces* species. Furthermore, a precise knowledge of the host range for each *Coelomomyces* sp. must be obtained prior to any field applications of the fungus. Ecological factors such as salinity tolerance, pH and temperature range requirements will be vital information for successful establishment of *Coelomomyces* where it is newly introduced.

Table 5: Emergence of cyclops from dry soil after flooding with tap water.

Hours after flooding	Nauphi	Adult	Adult + Egg-sacks.
	soil flooded with tap water		
0	—	—	—
16	—	—	—
17	—	—	—
18	—	—	—
19	—	+	—
23	—	+	—
40	—	+	+

Morphological Variation and Prevalence in the Cuticular Lesions on the Tsetse fly *Glossina pallidipes* (Glossinidae: Diptera)

M.O. Odindo, D.A. Tanner, E.D. Kokwaro, P.A. Amatalla, W.A. Otieno, D.M. Sabwa

Cuticular lesions (CLs) have been observed on six *Glossina* species caught in the field in Kenya, in *G. morsitans centralis* from Zambia, and in laboratory-reared *G. m. morsitans*. In the coastal area of Kenya, *G. pallidipes* populations show a high incidence of CLs. In the on-going investigation it was aimed to study the variation in the forms of lesions and to attempt to explain the differences in these variations.

Challier biconical traps were set up in four localities with varying vegetation types. Weekly samples were trapped in each area and examined under a Wild M 3 dissecting microscope. The tsetse bearing CLs were sorted on the type of lesion on the ventral abdominal cuticle, and photomicrographs taken on a Leitz Diloix E20 microscope. Tissues were also processed for scanning electron microscopy (SEM).

In SEM, tsetse abdomens were placed in 2.5% sodium cacodylate buffered glutaraldehyde and allowed to fix for 5 – 12 days and then dehydrated through ascending grades of alcohol to absolute ethanol. In order to remove surface debris, the abdomens were cleaned in the ultrasonic cleaner for 3 – 4 minutes. The wax coating of the cuticle was extracted for 4 hours with ethyl acetate in a soxhlet apparatus. Clean specimens were then double coated with carbon and gold in a vacuum evaporator and observed in a deol scanning electron microscope.

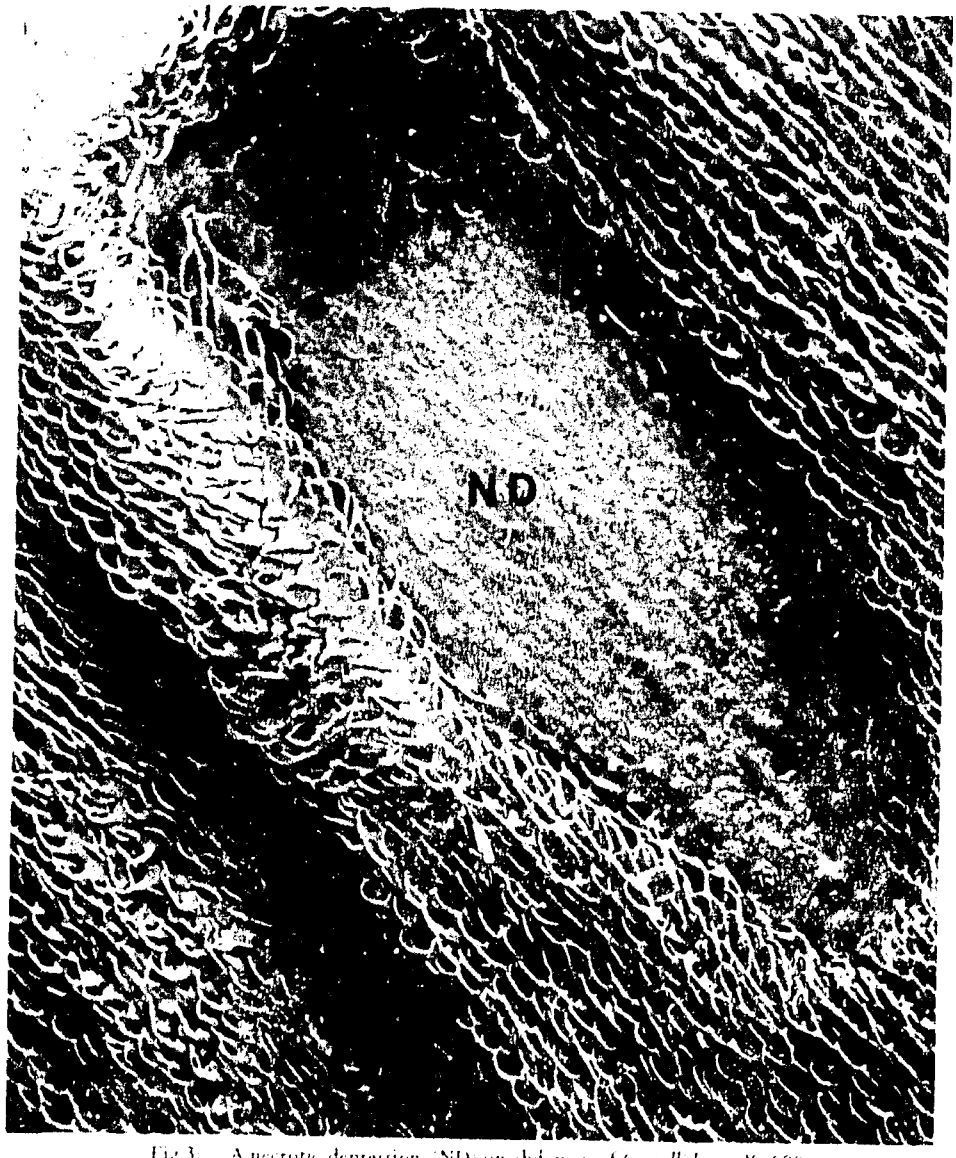

Fig 3 – A necrotic depression (ND) on abdomen of *G. pallidipes* X 600

Four kinds of lesions were observed on the ventral abdominal integument:

A definite depression on the cuticle forming a pit which was either shallow or deep. The edges were occasionally raised and the lesion often had a black shiny surface. Diameter 0.1 - 1.5 mm. These were referred to as necrotic depressions (ND) (Fig. 3).

A long scar varying from 0.1 to 3mm in length and often covering the whole length of the abdomen. These lesions occurred either singly or in groups. They were reminiscent of wounding of the cuticle referred to as emergence wounds (EW) (Fig. 5).

Fig. 4 A low power SEM micrograph of the abdomen of *G. pallidus* showing a large number of necrotic warts (NW) X 30

A raised warty growth found to be particularly associated with the upper abdomen over the crop and the spiracles. The shape of the warts varied from a small raised dark cuticle to an extensive "tumorous" growth rising 3 mm above the cuticular surface (Fig. 4). Though a few were porous, some were dark and solid. They were referred to as necrotic warts (NW).

An almost perfectly round and regularly shaped lesion with a dark central core. The central part of the lesion was extremely brittle and fell apart on firm pressure. The lesions were neither raised not depressed, and were called necrotic lesions (NL) (Fig. 6).

Fig. 5. An emergence wound (EW) running lengthwise on the abdomen of *G. pallidipes*. X 200

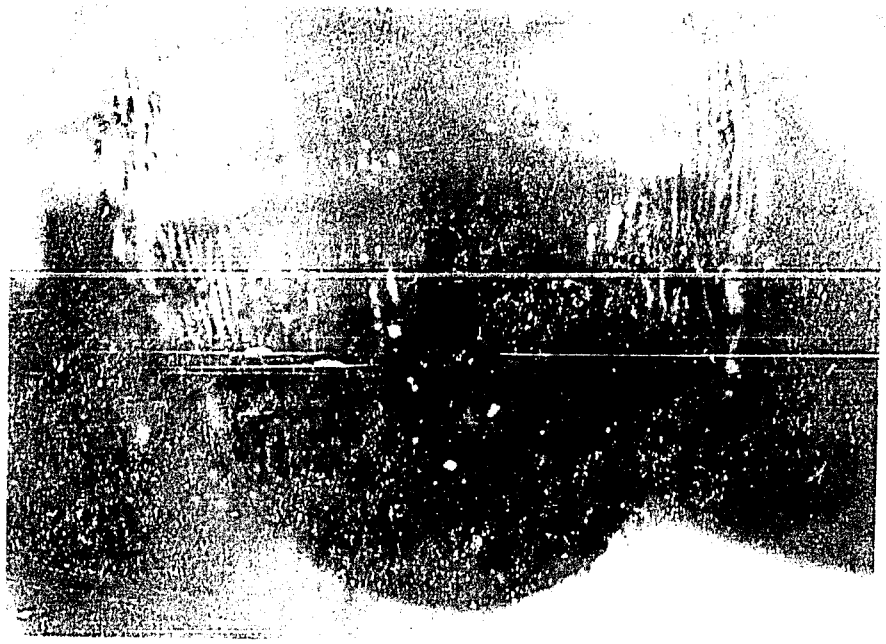

Fig 6 A photomicrograph of a necrotic lesion (NL) on the abdomen of *G. pallidipes* X 25

Field studies on the prevalence of the different lesions showed that the proportions of CLs on tsetse varied for the different trapping areas (Table 6), NL being the most prevalent CL in all areas. CLs were more common in female than male tsetse in all areas.

Work elsewhere has shown that three kinds of lesion (ND, NW, and EW) can be induced by teratological damage to the tsetse cuticle. It has also been shown that a suspension prepared by maceration of the cuticular lesions does not transmit infection to teneral flies when passed into the fly orally, by microinjection into tsetse haemolymph, or by topical application of the CL suspension. We therefore propose that ND, NW and EW are non-infectious teratologies caused to the insect in its normal habitat. The sites from which the flies were trapped were therefore important, for the prevalence of the lesion type.

Table 6 Incidence of cuticular lesions on *Glossina pallidipes* in four trapping sites.

Trapping site	Month	Total No tsetse		% tsetse bearing lesions							
				NL		ND		NW		EW	
		♂♂	♀♀	♂	♀♀	♂	♀♀	♂	♀♀	♂	♀♀
Shimba hills	May	727	1757	1.5	9.4	1.0	5.0	0.3	2.1	0.6	5.6
	June	250	250	3.0	10.0	1.6	7.6	0.6	1.0	1.0	7.2
	July	255	397	4.4	8.7	0.9	8.4	0.8	1.1	2.6	2.4
	Aug.	50	146	3.6	20.4	0.5	3.1	—	4.6	0.5	9.7
	Sept	194	193	4.9	11.9	1.0	10.6	0.5	0.5	0.3	2.8
	Oct.	119	281	4.1	15.6	1.3	11.8	0.3	1.8	2.3	5.1
Muhaka	June	24	50	—	8.1	—	8.1	—	—	—	9.5
	July	97	163	0.8	10.0	1.5	8.1	—	0.4	0.4	4.6
	Sept.	17	192	1.4	18.2	0.5	5.7	—	1.4	—	2.9
	Oct.	103	161	1.9	5.6	2.3	6.4	—	—	0.4	5.3
Diani Ukunda	July	187	312	4.0	6.8	1.4	5.4	0.8	1.8	1.8	6.0
	Aug.	172	175	2.3	9.2	1.2	1.2	0.9	1.7	2.6	6.3
	Oct.	136	212	5.2	11.2	2.0	6.6	1.1	2.3	1.7	9.8
Mwabungu	Aug.	160	260	3.3	10.2	1.0	1.2	0.2	3.1	1.2	3.3
	Sept.	14	143	3.7	10.2	2.1	10.2	0.5	0.5	2.7	2.7
	Oct	94	125	2.7	16.0	0.5	10.0	—	0.5	0.5	5.0

Briefly, the vegetation types of the four trapping areas were as follows:

Muhaka Forest is a relic of a typical rain forest with tall hard woods and thick undergrowth. The edges of the forest have been cut down and the secondary thicket supports low population of *G. brevipalpis* and *G. austeni* and slightly larger numbers of *G. pallidipes*. Muhaka had a low number of total CL count. For example in October CL count was 23.9% (N = 264).

Shimba Hills consists of tropical grassland interspersed with thick clumps of trees and occasionally, areas of thick rain forest. Parts of the natural habitat has been cleared and planted with soft woods. Both natural and planted forest support large numbers of *G. pallidipes*. Shimba Hills had a high CL count in October (42.2%, N = 391).

Ukunda — Diani and Mwabungu have a secondary vegetation of thicket and bush, mainly *Lantana*. CL count for October in these two sites were 39.9%, (N = 348) and 35.2%, (N = 219) respectively.

In the tests referred to above, formation of NL could not be induced by physical wounding. It was therefore proposed that NLs are caused by an attempt by parasitoids to parasitize the soft abdominal region of tsetse. The lesions were therefore thought to be the effect of toxins passed into the integument by parasitoids. There appeared to be remnants of an ovipositor in the centre of some lesions. Each attempt to penetrate the integument failed however, and the process was repeated thus leaving the characteristic pattern of NLs.

The investigations on cuticular lesions is ongoing, particularly the histo- and cyto-pathology of necrotic lesions.

Preliminary Tests on the Transmission of Virus-like Particles to *Glossina pallidipes* Austen (Glossinidae, Diptera)

M.O. Odindo, D.M. Sabwa, P.A. Amutalla

Data accruing from observations on the frequency of salivary gland hypertrophy (HSG) in field populations of *Glossina pallidipes* have shown the prevalence of HSG in very young teneral flies (ovarian category Oa and Ob). However, it has has also been observed during the same investigations that tsetse flies in ovarian category 4, 5 and 6 also showed early stages of HSG. Hence whereas the former observations supported trans-ovarian and/or transovum transmission of virus-like particles (VLPs), the latter must mean that *G. pallidipes* can pick up the infection during their life-time, resulting in hypertrophy of the salivary glands.

Intrahaemocoelic Infection

A 10 µl Microcaps (R) micropipette was graduated so as to deliver 1. µl aliquot suspension. Microinjectors were prepared by heating one end of the micropipette over a bunsen burner and drawing it out to form a thin and extremely sharp needle (<0.01 mm diameter). The microinjector was attached to a dispenser (a rubber bulb with tube supplied by Drummond Scientific Co.) and used to draw 3 µl virus suspension, which had been purified by differential centrifugation and membrane filtration.

Teneral *G. pallidipes* were injected into the hollow at the tip of the V formed by the sutures between coxa II and III. Sixty-five tsetse were thus infected by intrahaemocoelic pathway with 2 µl virus suspension.

Oral Infection

A second group of 65 flies were infected *per os*. A 10 µl micropipette was used to draw a virus

Table 7 Salivary gland hypertrophy in *Glossina pallidipes* infected with Virus like particles by two pathways of infection.

No. days post infection	No. tsetse flies		Cumulative % infection	
	MI[1]	OI[2]	MI	OI
0	37	29	(3.6)[3]	(3.6)[3]
7	18	16	5.5	6.2
10	14	13	5.5	6.2
13	9	8	16.7	18.8
16	6	4	27.8	25.0
19	2	1	38.9	31.3
22	—	—	44.4	31.3

Notes: 1 Infection by microinjection
2 Infection by oral feeding
3 Natural virus like particle infection in population

suspension, and holding a tsetse between the thumb and index finger of the left hand, and a micropipette with the right, the proboscis was inserted into the end of the micropipette so that only the tip of the fascicle was dipping into the liquid. Flies took up the liquid better when the free end of the micropipette rested on the side of the fascicle rather than when dipping directly into the liquid. Tsetse took up 0.5 µl of virus suspension. The test tsetse were fed ad lib on rabbit ears and reared at 27° ± 2°C and 80 – 90% R.H., and samples of tsetse were dissected for 3 weeks.

Flies with HSG were observed from 7 days post-infection in both intra-haemacoelic and *per os* infected flies. When all flies infected had been dissected at 3 weeks 44.4% (N = 37) of the tsetse infected through the intra-haemocoelic pathway and 31.3% (N = 29) of flies infected *per os* were found to have HSG (Table 7). The transmissibility of VLPs in the field was thus established.

Cuticular Lesions : A Non Infectious Integumental Disease on *Glossina* species

M.O. Odindo, D.A. Turner, W.A. Otieno, D.M. Sabwa, P.A. Amutalla

Cuticular lesions (CLs) and tumorous growths of various forms are known to occur in insects, and may be associated with nuclear polyhedrosis virus infections (*Pseudoletia unipuncta*, *Hyphantria cunea*). Previous investigations had shown that six Glossina species had lesions in the abdominal integument – *G. pallidipes*, *G. swynnertoni*, *G. austeni*, *G. fuscipes*, *G. brevipalpis* and *G. longipennis*. Further work was designed to investigate the infectious and contageous nature of the lesions in *G. morsitans*.

Preparation of Lesion

The tsetse flies *G. morsitans*, bearing four kinds of lesions – necrotic lesion (NL), necrotic depression (ND), necrotic wart (NW) and emergence wound (EW) – were washed in water and surface sterilized in 1% sodium hypochlorite solution for 7 minutes. Tsetse bearing similar lesion type were sterile dissected and the CL carefully incised off. The lesions were placed in sterile distilled water in a specimen tube and macerated to form a fine suspension. The suspension was filtered through muslin cloth to preserve any bacterial or protozoan cells, fungal spores or mycelia/hyphae and virus particles.

Infection of Tsetse Flies

Newly emerged teneral flies were divided into four groups (32 tsetse each) and injected with 2 µl of the purified NL, ND, NW, or EW suspension. A fifth group of tsetse were infected orally using Microcaps (R) micropipettes (Drummond Scientific Co., USA). To a sixth group of tsetse 5 µl of the CL suspension was applied topically on the cuticle.

Physical Induction of Cuticular Lesions

Neonatal teneral tsetse were numbed at 2°C and three kinds of wounds made on the ventral abdominal cuticle to simulate the different kinds of lesions observed on the tsetse cuticle.

The tsetse flies were examined every week for 11 weeks and the type of lesion forming on the cuticle noted. A sample of tsetse was also dissected from each group each week and the salivary gland examined for hypertrophy.

There were no CLs on tsetse flies infected *per os* by topical application. All tsetse on which CLs were induced by wounding the integument formed either ND, EW or NW types of lesions depending on the nature of wounding (Table 8). NLs were not formed by physical induction, topical application, microinjection or feeding on CL suspension. None of the dissected tsetse had the salivary gland hypertrophied. The evidence from these investigations show that CLs on *Glossina* are teratological in origin, and are non-infectious and non-contageous.

Table 8: Cumulative number of tsetse flies *Glossina morsitans* showing cuticular lesions at 3, 28, and 77 days post-treatment

Time	Treatment					
Days post infection	Infection			Induction		
	Inj 1	Oral	Topical	Stab	Slit	Pinch
3	ND[2]	–	–	11 ND	13EW[3], 2ND	12NW[4], 4ND
28	ND	–	–	11 ND	13EW, 2ND	12NW, 4ND
77	ND	–	–	11 ND	13EW, 2ND	12NW, 4ND

Note
1. Tsetse flies microinjection in the abdomen all bore lesions at point of entry of needle.
2. ND = necrotic depression.
3. EW = emergence wound
4. NW = necrotic wart

Gut Microflora of Maize Stem-borer, *Chilo partellus* (Swinhoe)

D.M. Sabwa, W.A. Otieno, Z.T. Dabrowski, P.A. Amutalla

Research continues on the gut microflora species composition of *Chilo partellus* larvae and its possible role in the resistance of maize and sorghum plants to the borer's attack. Dissections have been carried out on 80 larvae, revealing a wide range of bacterial types and on yeast species. Some have been identified to the species level, and comparisons done on the differences in species composition between maize and sorghum larvae. The consistency of occurrence of the different isolates has also been determined.

Under sterile conditions, live larvae from the field were anaesthetized with chloroform, and the oral and anal openings sealed. They were surface sterilized in 5% sodium hypochlorite, the gut aseptically dissected out and crushed in saline. The suspension was then immediately cultured in Sabouraud dextrose agar (SDA), Nutrient agar (NA) or Brain heart Infusion (BHI) plates. These were incubated at 35°C under aerobic conditions. After 48 hours, the colonies were cultured. Identifications were done from colony growth characteristics, morphological appearances and various biochemical tests. (Table 9)

Ten different bacterial types and a yeast have been isolated. The most frequently occurring organism being the yeast which has been isolated in all but one of the insects dissected.

Pseudomonas aeruginosa: Small gram-negative rods occur singly and are not spore-formers. Form a greenish pigment on agar, which later turns pinkish brown.

Serratia marcescens: Short, motile rod-shaped cells that are sometimes almost spherical. Agar colonies are bright red in colour.

Bacillus group: These are gram positive rod-shaped bacteria of varying sizes that may occur singly or in chains. Many are spore-formers and grow well on nutrient agar under aerobic conditions.

Cocci group: Gram positive spherical cells occurring singly, in two's chains or in clusters.

Yeast (*Candida* spp): A budding yeast that grows very well on SDA media with hyphae. Tentatively identified as *Candida* sp.

Table 9: Identification tests for *Chilo partellus* gut microorganisms

Test on Substrate	*Pseudomonas aeruginosa*	*Bacillus cereus*	*Serratia marcescens*	*Bacillus subtilis*
Gram reaction		+	–	+
Growth				
at 42°C	+			
pH 5.7		+		+
in lysozyme (.001%)		+		–
in 7% NaCl		+		+
anaerobically	–	+		–
Use of				
Citrate	+	+	+	+
Propionate		–		–
Glucose	+ (acid)	+ (acid)	+ (acid+gas)	+ (acid)
Lactose		–	–	
Sucrose		–		
Xylose	+ (acid)	–		+ (acid)
Arabinose		–	–	+ (acid)
Galactose		–		
Adonitol			+ (acid)	
Sorbitol			+ (acid)	
Dulcitol			–	
Maltose		–		
Inositol		–		
NO_3 to NO_2		+		
Catalase	+	+		+
Urease	+		+	
H_2S production	–		–	
Indole	–		+ (weakly)	
Acetylmethyl carbinol			+	
Pyocyanin	+			
Starch hydrolysis		+		+
Voges Proskauer		+		+
Egg yolk test		+		–
Diffusible pigment	+			+
Oxidase	+			

Table 10: Frequency of occurrence and distribution of microorganisms in larvae of *Chilo partellus* from maize and sorghum plants

Isolate	Frequency of occurrence (n = 80)	Distribution in maize larvae (n = 28)	Distribution in Sorghum larvae (n = 52)
Pseudomonas aeruginosa	35	46%	42%
Serratia marcescens	3	0%	5.8%
Bacillus subtilis	12	17%	13%
Bacillus cereus	9	17%	7%
Flavobacterium sp	7	14.3%	5.8%
Staphylococcus sp.	30	28%	42%
Streptococcus sp	10	14.3%	11.5%
Micrococcus sp	31	46%	34.6%
Bacillus sp	10	25%	5.8%
Bacillus sp 2	11	17%	11.5%
Candida sp	79	100%	98%

Maize and sorghum appear to have fairly uniform and similar bacterial types (Table 10). No larva has been found sterile or to harbour more than four bacterial types at the same time.

The variety of microorganisms isolated is quite wide but the majority occur so infrequently that they could hardly be considered to be of any possible significance to larval development. Some potentially pathogenic bacteria have been isolated although they do not seem to cause mortalities in the larvae when ingested to the gut. Due to the inconsistent occurrence of most bacterial types and the presence of potentially pathogenic strains, it is likely that most of these microorganisms are introduced into the gut by chance through feeding from the larval environment. These are not likely to be of any beneficial significance in larval development. Some Coccus groups have been implicated in other studies as possible permanent residents of the guts of some lepidopteran larvae. These require long anaerobic isolation methods which have not yet been employed in this study. Possibly when these anaerobic methods are employed, such bacteria types may be recovered and analysed for their significance in the gut.

Defence Reactions of some Medical Vectors to Pathogens and Other Foreign Materials

G.P. Kaaya

Many insects have been shown to possess the capacity to defend themselves against invading pathogens. The mechanisms of defence are not well understood, especially in the order Diptera, where many species are vectors of important tropical diseases such as malaria, trypanosomiasis, leishmaniasis and filariasis.

A thorough understanding of Dipteran defence mechanisms would enable scientists to develop the capacity to manipulate these defences and thus interrupt the infection cycle in important parasitic diseases. Investigations into the defence mechanisms of the tsetse fly and mosquitoes were started a year ago and some preliminary results are given below.

Tsetse Flies (*Glossina morsitans* and *G. austeni*)

Tsetse flies have very low susceptibility to trypanosome infections, both under field and laboratory conditions. Furthermore, newly emerged tsetse flies are more susceptible to infection than are older flies. This research project has aimed at determining what role the tsetse defence mechanisms play in the establishment of trypanosome infections. Before embarking on this major project, simple experiments were conducted to determine how tsetse flies respond to bacterial infection and to implantation with foreign tissues.

Newly emerged *Glossina morsitans* and *G. austeni* were given intrahemocoelic injections of heat-killed or live *Bacillus cereus* at a dosage of 1×10^6 bacteria per fly. The flies were then bled at various time intervals in order to investigate the interaction of hemocytes with the bacteria.

All the flies injected with live *B. cereus* died within 3-5 hours post injection. At the time of death, several plasmatocytes (phagocytic hemocytes) contained intracellular bacteria (Fig. 7). Flies injected with heat-killed bacteria survived for several days. Twenty-four hours post injection, most of the plasmatocytes were observed to contain intracellular bacteria. Occasionally, several plasmatocytes were observed to have fused together forming "giant cells" containing chains of intra-cytoplasmic bacilli (Fig. 8).

A continuous decrease in the hemolymph bacterial concentration was also observed (Fig. 9). Nodule formation was not observed in *Glossina* injected with *B. cereus*, although the same bacteria induced nodule formation in larvae of *Galleria mellonella*. These nodules were easily visible with the dissecting microscope and appeared as small black dots.

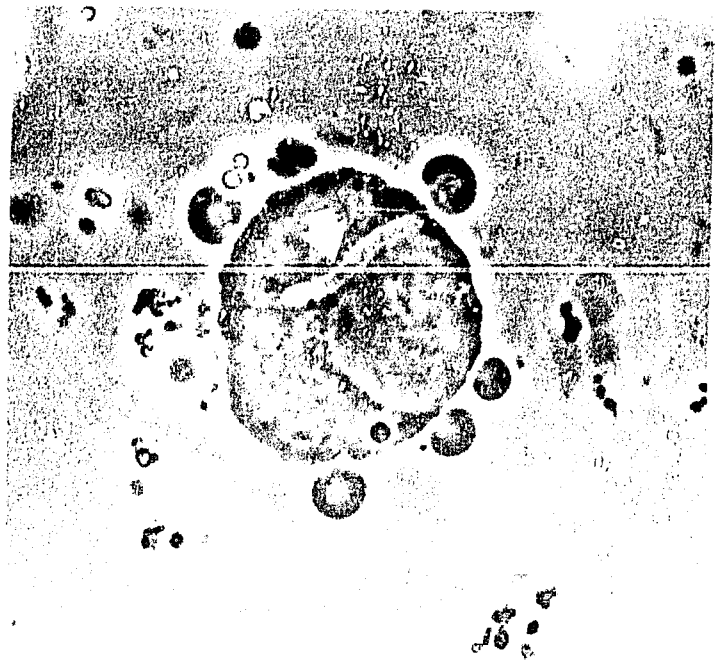

Fig 7: A plasmatocyte from *G. mor itans* containing intracellular bacillus (arrow). Magnification X 1,800 (phase contrast)

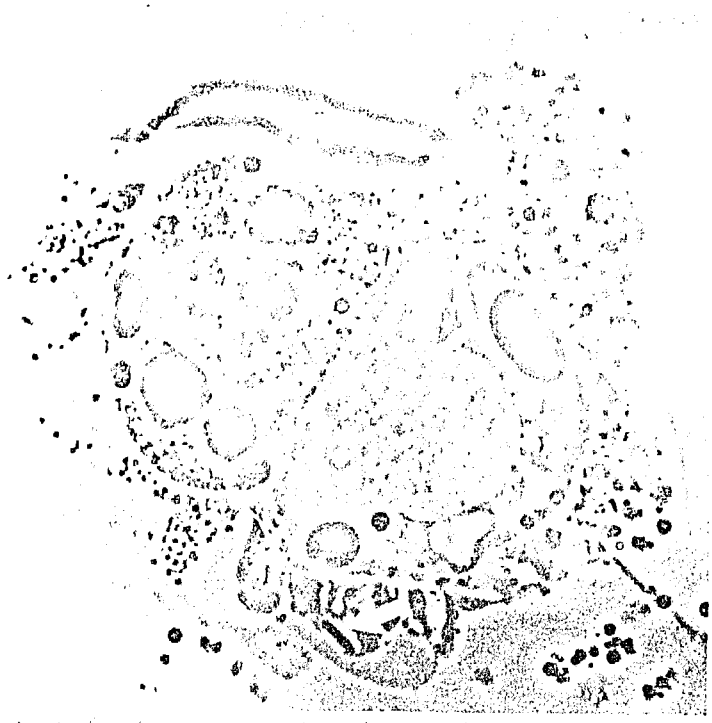

Fig 8: Fixed plasmatocytes from *G. mor itans* containing phagocytized bacilli, some in short chain (arrows). Magnification X 1,300 (phase contrast)

Muscle fragments of *Calliphora* and *Locusta* were implanted in the hemocoels of several *G. morsitans*. These fragments were retrieved at various time intervals and studied histologically to determine the type and extent of defence response. Muscle fragments retrieved after 48 hours showed moderate to heavy melanization of the implants, as well as the neighbouring tissues (Fig. 10). This form of encapsulation (melanization) in which other insects e.g. in larvae of *G. mellonella*, a foreign implant is quickly encapsulated by a multicellular sheath of hemocytes within 24 hours after implantation. Cellular encapsulation was not observed in this experiment.

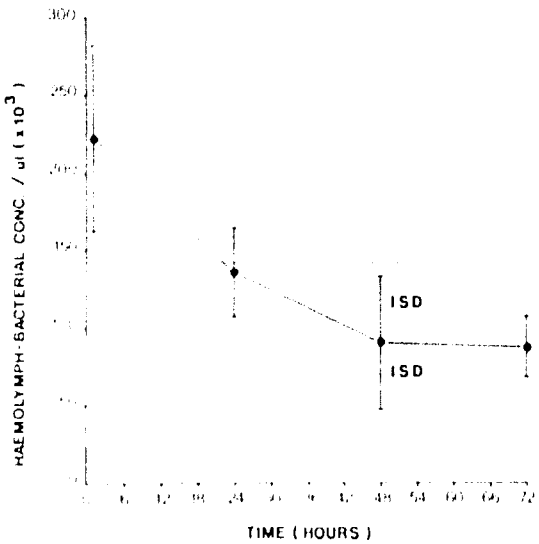

Fig 9: Rate of hemolymph clearance of *B. cereus* in young *G. morsitans*.

(encapsulated) microfilariae. This phenomenon of resistance was investigated in *A. triseriatus* and in *A. aegypti* infected with the dog filaria, *Dirofilaria immitis*, which develops in the malpighian tubules of mosquitoes.

In the refractory strains of mosquitoes, most of the microfilariae degenerated in the malpighian tubules of mosquitoes without direct participation of hemocytes or even melanization. However, a few microfilariae were observed to be partially or completely encapsulated in a hard, melanized, sheath. Melanin deposition was observed to begin at either end of the microfilariae, at the middle region, or even at various regions (Fig. 11). Hemocytes were not observed to be directly involved in this process of melanization. Various histochemical tests for melanin failed to reveal presence of melanin sheaths on the surfaces of most of the degenerating microfilariae, suggesting that other humoral factors are involved in this degenerative process.

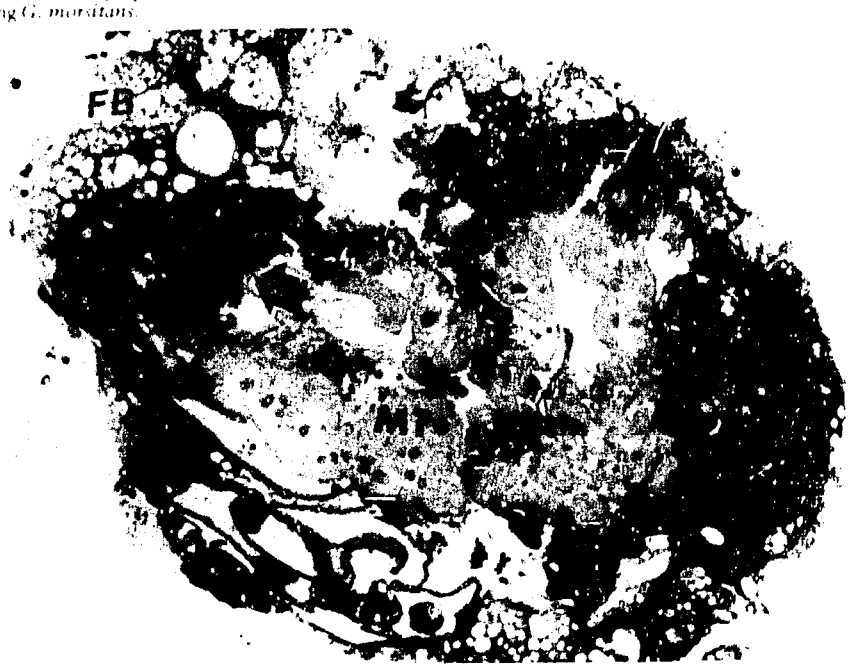

Fig 10: A *Locusta migratoria* muscle implant in hemocoel of *G. morsitans*. Notice the heavy melanization of the two visible portions of the implant (arrows), and of the surrounding fat body (FB). Malpighian tubules (MT) are entangled in the terminal capsule. Magnification X 1,500.

Mosquitoes (*Aedes triseriatus* and *A. aegypti*)

Some species and even strains of mosquitoes have been shown to vary significantly in their susceptibility to filarial infections.

It has been shown that susceptibility of mosquitoes to filarial parasites is controlled by a sex-linked recessive gene, and that it is possible to establish very susceptible and very refractory strains by applying artificial selection pressure.

The chemical mediators of this resistance to infection are unknown. However, in the refractory mosquitoes, it is not unusual to find melanized

Fig. 11. A melanized larva of D. immitis in a malpighian tubule (MT) of *A. triseriatus*. Notice three foci of melanization (arrows). Magnification X 400.

BIOASSAY RESEARCH UNIT

Research Staff

Dr. T. Gebreyesus (1978) Research Scientist
Mr. B.N. Odero (1976) Chief Technician
Mr. E.N. Ole Sitayo (1979) Senior Technician
Mr. G. Achieng (1976) Technical Assistant
Mr. L. Moreka (1976) Technical Assistant

Services Provided By the Unit

T. Gebreyesus

The Bioassay Research Unit continues to provide routine bioassay services to the different Programmes and Units at the Centre. Within the limits of staff and space, the unit has tried to widen the number of bioassays it provides. Thus a number of the assays experimentally established previously such as the fumigation tests for anti-juvenile hormone activity and the larvicide tests are now offered routinely. In addition an antifungal assay using *Cladosporium cucumerinum* has been established.

The test involves taking the plant extract or other compound to be tested, spotting it on a commercial silica gel thin layer plate, developing it on a suitable solvent and spraying the plate (after drying) with the fungal spores suspended in tomato juice; the plate is incubated in a plastic bag (kept moist by wetted paper towels) at room temperature and observed after 24 and 48 hours. The fungus appearing somewhat black, grows all over the plate except where an antifungal compound occurs.

Antifeedants and Fungicides from *Clausena anisata* (Rutaceae)

T. Gebreyesus

During the screening of plant extracts for antifeedants using African armyworm *(Spodoptera exempta)* larvae in the leaf disc bioassay, the petroleum ether extract of *Clausena anisata* (Rutaceae) was found to be active. The extract also showed fungicidal activity against *Cladosporium cucumerinum*.

The active compounds were isolated and purified by column and thin layer chromatography. Two compounds had feeding deterrence activity at 100 and 500 parts per million. These compounds were characterized from their physical and spectral properties as the known coumarins imperatorin and xanthoxyletin respectively. The identification was confirmed by gas and thin layer chromatography comparisons with authentic samples kindly supplied by Drs. Dreyer and Mester. Another coumarin 3–(1, 1–dimethylallyl)–xanthyletin, isolated from the same plant did not show feeding deterrence activity.

Xanthoxyletin also shows antifungal activity against *Cladosporium cucumerinum*. Other minor components in the same extract show fungicidal activity. Their isolation and identification is in progress.

INSECT AND ANIMAL BREEDING UNIT

Research Staff

Mr. A.J. Leaney (1980) Controller for Insectary Services
Mr. J. Wanyonje (1970) Principal Technician
Mr. H. Bandah (1972) Technician
Mr. J. Kagoiya (1973) Technician
Mr. A.S. Ikhunyalo (1971) Junior Technician
Mr. J. Ongudha (1973) Junior Technician
Mr. E.O. Awuoche (1973) Junior Technician
Mrs. R. Kariuki (1974) Technical Assistant
Mr. G.M. Birir (1978) Technical Assistant/Driver

Breeding of Tsetse Flies

A self sustaining colony of *Glossina morsitans* was maintained throughout 1980, using rabbits as host animals, at a temperature of 25°C ± 1°C and R.H. 70% ± 10%.

Tsetse emergence per month ranged rom 6287 ♂♂ and 6422 ♀♀ to 8033 ♂♂ and 8391 ♀♀. The mean monthly emergence being 7,091 ♂♂ and 7,227 ♀♀. Mortality in teneral flies was negligible and in mated ♀♀ never exceeded 1.1% per day. (mean mortality was 0.55%).

The mean pupal production per female per month was 2.67, and samples of pupae weighed throughout the year gave a mean pupal weight of 29.03 mg.

A total of 13,345 ♂♂, 16,415 ♀♀ and 1909 pupae were supplied for experimental use to ICIPE scientists and other research organisations.

Breeding of *Chilo partellus*

At the beginning of 1980 a small *Chilo* colony was maintained on artificial diet in an insectary shared with other, different, species of insects. Conditions were not ideal and high mortality of first instar larvae and delay in pupation were experienced. The colony was transferred to a newly completed insectary in August, but improvement in environmental conditions did not improve the colony. A wheatgerm diet using newly purchased materials and incorporating a reduced amount of aureomycin (0.07g per 500 ml. as opposed to 0.36 g) was introduced in November and has alleviated the problems. Mortality in first instars has become slight and the normal larval duration period of 21 days has been restored. However, the diet deteriorates and more frequent changing is necessary. The colony at present stands at 1500 larvae of all stages and expansion is envisaged.

Breeding of African Armyworm – *Spodoptera exempta*

Late in 1979 sterilisation procedures were introduced for eggs and pupae. Washing eggs in 0.1% sodium hypochlorite and 10% formaline, and pupae in 0.1% sodium hypochlorite increased hatching, and the presence of virus, a constant problem in the colony, declined.

Infection in the colony reappeared in March, both viral and bacterial and was probably caused by contamination from field material kept temporarily in the breeding insectary. The colony recovered but declined in August due to chemical spillage in a room adjacent to the insectary. The colony has again reached its former level and despite these problems 6000 – 12000 larvae hatched per month throughout 1980 and the insectary has supplied 13,255 late instar larvae, 566 pupae and 514 adults for research purposes both within and outside the ICIPE.

Animal Breeding

Rabbits

At the start of the year the rabbit colony was small and unable to supply the needs of the ICIPE scientists. Rabbits were purchased from elsewhere. They were largely of unknown history and suffered high mortalities.

In March 1980 the number of breeding females was increased to 120 grouped into 6 batches of 20. Two batches were mated during the first two weeks of March, April and May. Females were not mated again until the week after the weaning of current litter. The period from mating to weaning is approximately 3 months, so by the end of August all the females had been given the opportunity of producing 1 litter. The 120 females produced 608 young of which 514 survived to weaning (85%). This represented a 6% increase in the survival of young rabbits. The availability of a choice of healthy animals reduced the mortality in rabbits, used as hosts for tsetse, from 75% per month in January to 17% in October and consequently the colony was producing in excess of the demand.

The number of breeding females has therefore been reduced to 90, mated according to the same system. Survival in young rabbits has increased to 90% and currently the colony meets the requirements for tsetse feeding and supplies 60 – 80 rabbits per month for other experimental purposes.

Rodents

The demand for rodents has increased during 1980 and the colonies have been expanded accordingly. Currently up to 75 rats and 100 mice per month are supplied for research.

MAJOR SEMINARS GIVEN AT THE ICIPE DURING 1980

Speakers	Subject
1. Dr. L. Tieszen Augustana College, Sioux Falls, U.S.A.	The use of Stable Isotope Ratios in the Study of Insect Behaviour
2. Professor S. Yagi Tokyo University of Agriculture and Technology	Hormonal Control of Larval Diapause and Phase Variation in some Lepidopterous larvae
3. Dr. T. Golder ICIPE	Non-neural Cholinesterase in the Tsetse Fly *Glossina morsitans*
4. Miss L.M. Oketch ICIPE	Mosquito Identification
5. Dr. David Kemp C.S.I.R.O. Long Pocket Laboratories, Brisbane, Australia	Feeding of the tick *Boophilus microplus* and Expression of Host Immunity
6. Dr. F.L. Lambrecht ICIPE	Ecological Epidemiology Study in the Meru National Park
7. Dr. M. Cunningham ICIPE	The Tick Programme An Overview
8. Mr. F.M. Mwega University of Nairobi	An Economic Analysis of Damages Caused by Ticks and Tick-borne Diseases, particularly in relation to East Coast Fever
9. Dr. F.D. Obenchain ICIPE	Successes and Failures in Recent Studies on Ticks
10. Dr. C.J. Den Otter ICIPE	Sensory Physiology and Insect Behaviour
11. Dr. Douglas Light Max-Planck-Institut for Verhaltensphysiologie	Olfactory Chemoreception of Behaviour Modifying Chemicals in IPS (Scolytidae)
12. Professor G.B. Marini-Bettelo Universita Cattolica Del Sacro Cuore, Rome	Chemical Studies in African Plants
13. Dr. David G. Furth The Hebrew University of Jerusalem	Aspects of Herbivore Co-evolution
14. Dr. R. Newson ICIPE	Host Effects on Tick Populations
15. Dr. L.R.S. Awiti ICIPE	Ultrastructural Studies on the Mesadenial Accessory Reproductive Gland its role in Reproduction in *Dysdercus fasciatus* (Signoret)

16. Dr. G.B. White Entomology Department London School of Hygiene and Tropical Medicine, U.K.	The *Anopheles gambiae* Complex : Vector Functions in Relation to Malaria and Filariasis
17. Dr. R.S. Pathak ICIPE	Cowpea Improvement in Kenya
18. Dr. G.B. White Entomology Department, London School of Hygiene and Tropical Medicine, U.K.	Steps Towards a Solution of the *Culex quinquefasciatus* Problem
19. Dr. Donald Pickering World Bank Headquarters, Washington, D.C.	The World Bank and Rural Development
20. Dr. A. Hassanali University of Dar-es-Salaam Tanzania	A Novel Approach to the Control of Parasitic Weeds
21. Dr. G.P. Kaaya ICIPE	Morphological Classification of Insect Haemocytes
22. Dr. S. Nielson Director International Federation of Institutes for Advanced Study	The Second Industrial Revolution — North : South
23. Dr. Wendy Gibson K.E.T.R.I. Muguga	Brucei Group Trypanosomes: Isoenzymes Taxonomy and Epidemiology
24. Dr. Hans G. Hamann Society of Radiation and Environmental Research, Hannover	Experiments on the Suitability of Radio-Sterilization of Blood for *in vitro* Feeding of Tsetse Flies
25. Professor B.J.R. Philogene Department of Biology, University of Ottawa	Diapause and the Light Requirements of Insects
26. Dr. M.A. Altieri Department of Entomology University of Georgia	Manipulation of Insect Populations through Vegetation Management
27. Dr. David A. Carlson U.S. Department of Agriculture, Florida	Tsetse Sex Stimulant Pheromones, Species Specific Aphrodisiacs for Male *Glossina morsitans morsitans* and Implications for use in the Field
28. Dr. G.P. Kaaya ICIPE	Insect Defence Mechanisms, with Special Emphasis on Dipterans
29. Dr. F. Owino Department of Forestry University of Nairobi	The Implementation of more Quantitative Research at ICIPE
30. Dr. J.V. Clark ICIPE	Studies on the Feeding of *Spodoptera exempta*
31. Dr. P.V.M. Mahadev London School of Hygiene and Tropical Medicine	Some Observations on *Aedes aegypti* Distribution and Prevalence in India

SEMINARS AT MUHAKA FIELD STATION

Speakers	Subject
1. Prof. Thomas R. Odhiambo	Management, Research and Development Activities of the ICIPE
2. Prof. R. Galun	Romantics of the Dipteran Flies
3. Prof. P.S. Corbet	Behaviour and Ecology of the Dragonflies
4. Dr. David Furth	Aspects of Herbivore Co-evolution
5. Prof. A.S. Tahori	Research Activities at the ICIPE
6. Dr. P.V. M. Mahadev	Some Observations on *Aedes aegypti* in Maharashtra State, India
7. Prof. F. Matsumura	Objectives of UNDP Mission to the ICIPE
8. Dr. R. Subra	Competition between *Culex quinquefasciatus* and *Culex cinereus*
9. Dr. W. A. Otieno	A Fungus, *Coelomomyces indicus*, a Naturally Occurring Mosquito Pathogen
10. Dr. A.W.R. McCrae	Oviposition and Aquatic Ecology of *Anopheles gambiae* complex
11. Dr. G.P. Kaaya	Morphological Classification of Insect Hemocytes
12. Dr. W.E. Snow	The Ecology of *Glossina pallidipes* on the Kenya Coast
13. Dr. M.O. Odindo	The Virus-like Particles Associated with the Tsetse flies
14. Dr. F.W. Mosha	Ecology of *Anopheles merus*
15. Miss Lucy Olyetch	Mosquito Taxonomy
16. Miss Diana Sabwa	The Gut Microflora of Maize Stem-borer, *Chilo partellus*
17. Dr. G.P. Kaaya	Dipteran Hemocytes and their Role in Defence Mechanism.
18. Dr. P.S. Corbet	Pest Control and Environmental Management
19. Mr. S.R. Tarimo	Patterns of Trypanosome Infection in *Glossina pallidipes* on the Kenya Coast

SEMINARS AT MBITA POINT FIELD STATION

Speakers	Subject
Mr. J.B. Okeyo Owuor	Basic Principles of Crop Protection
Mr. T. Omulo	Some Observations on the Biology of the Cowpea Pod Borer *Maruca testulalis* Geyer at Mbita Point Field Station
Mr. P.O. Odinga	Experimental Ecology of Crop Pests
Mr. J.C. Olela	Responses of Sorghum Shootfly (*A. soccata*) to various colours at ICRISAT, India
Mr. J.J. Njokah	Assessment of Yield Loss due to Insect Damage at Four Growth Stages of Irrigated Lowland Rice
Mr. E.O. Omolo	Experimentation

LIST OF PUBLICATIONS

This list has been compiled for the years 1972–1981. It contains papers published in scientific journals and/or in proceedings of conferences, and is by no means an exhaustive one. However, it represents most of the work done by ICIPE staff members. It is ICIPE's intention to update this list annually.

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