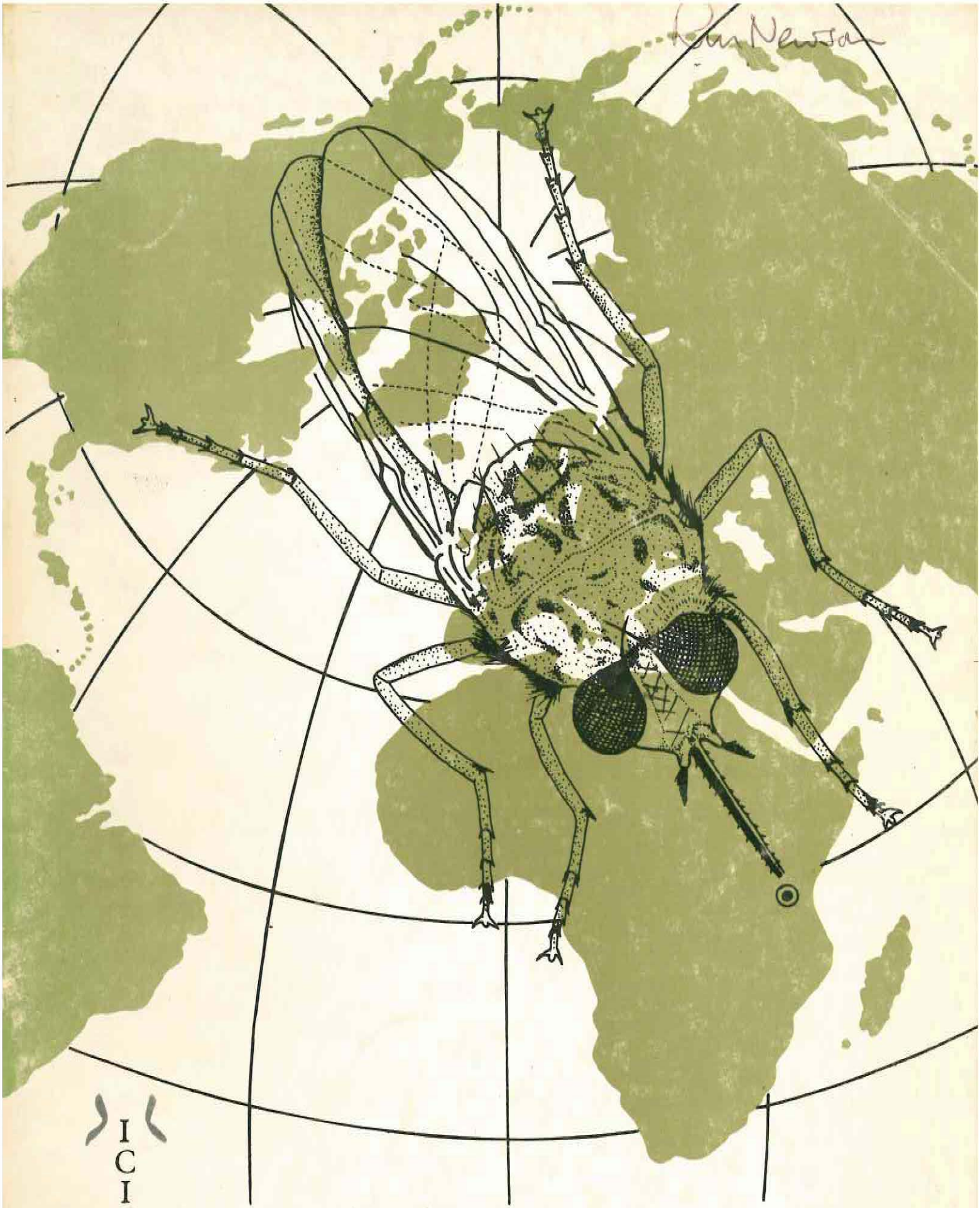

ICIPE

FIFTH ANNUAL REPORT — 1977

THE INTERNATIONAL CENTRE OF INSECT PHYSIOLOGY AND ECOLOGY

FIFTH ANNUAL REPORT

1977

Nairobi, March 1978

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Professor J. M. Mungai, Department of Anatomy, University of Nairobi, Nairobi, Kenya
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ICIPE GOVERNING BOARD (from July, 1977)

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Royal Swedish Academy of Engineering Sciences
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Czechoslovak Academy of Sciences
Dr. Vladimir Landa

Academy of Finland
Professor H. G. Gyllenberg

African Committee Representatives
Professor A. S. Msangi
Professor T. Ajibola Taylor

The International Committee has been replaced by the
ICIPE Foundation as from September 1977

VISITING DIRECTORS OF RESEARCH

Insect Ecology and Genetics
- Dr. W. Helle (1970), Universiteit van Amsterdam, Netherlands
- Dr. W. A. Sands (1973), Centre for Overseas Pest Research, London, United Kingdom

Insect Sensory Physiology and Behaviour
- Professor A. R. Møller (1973), University of Goteborg, Sweden
- Professor D. Schneider (1970), Max-Planck Institute, Seeweisen, West Germany
- Professor J. W. S. Pringle (1970), Department of Zoology, University of Oxford, United Kingdom

Insect Pathology and Pest Management
- Dr. L. Brader (1975), FAO, Rome

Insect Histology and Fine Structure
- Professor M. Locke (1977), University of Western Ontario, Canada

Insect Endocrinology
- Dr. W. S. Bowers (1977), Cornell University, Ithaca, New York, USA
- Professor M. Lüscher (1970), University of Bern, Switzerland
- Professor T. R. Odhiambo (1970), ICIPE Research Centre, Nairobi, Kenya

Insect/Hostplant Relations
- Professor L. M. Schoonhoven (1975), University of Wageningen, Netherlands
- Professor K. N. Saxena (1977), University of New Delhi, India

Vector Biology
- Professor R. Galun (1970), Israel Institute for Biological Research, Israel
- Dr. J. Mouchet (1975), ORSTOM, France
- Dr. A. R. Njogu (1975), Trypanosomiasis Research Organization, Kenya

Insect Biochemistry and Natural Products Chemistry
- Professor O. L. Chapman (1977), University of California, Los Angeles, USA
- Professor J. H. Law (1977), University of Chicago, Chicago, USA

RESEARCH SCIENTISTS

			Appointed
African Armyworm Research			
	Dr. S. Khasimuddin	India	1.12.73
	Dr. B. I. P. Persson	Sweden	1. 9.77
	Dr. D. J. W. Rose	United Kingdom	1. 7.77
Sorghum Shootfly Research			
	Dr. J. R. Clearwater	New Zealand	11.7.75
	Dr. A. K. Raina	India	1.9.77
Mosquito Research			
	Dr. R. Subra	France	29. 9.75
	Dr. F. Ogah	Nigeria	1.10.73–28.9.77
	Dr. A. W. R. McCrae	United Kingdom	1.11.77
Termite Research			
	Dr. N. Abo-Khatwa	Egypt	15.11.73–30.9.77
	Dr. M. A. Arshad	Canada	15. 7.77
	Ir. O. H. Bruinsma	Dutch	1.11.74
	Dr. G. Bühlmann	Switzerland	1. 4.75

Dr. J. P. E. C. Darlington	United Kingdom	1.10.74
Dr. T. Fukushi	Japan	1. 7.76–31.3.77
Dr. M. G. Lepage	France	24. 7.75
Dr. G. W. Oloo	Kenya	1. 5.74

Livestock Tick Research

Dr. M. P. Cunningham	United Kingdom	1.10.77
Dr. R. M. Newson	United Kingdom	1. 9.74
Dr. F. D. Obenchain	U.S.A.	6.10.76

Tsetse Salivary Gland Physiology

Dr. M. B. A. Nyindo	Tanzania	1.12.76
Dr. L. H. Otieno	Kenya	1. 2.73

Tsetse Reproductive Physiology

Dr. M. F. B. Chaudhury	Bangladesh	1. 3.74
Dr. K. Endo	Japan	1. 6.77

Tsetse Population Diversity

Dr. W. A. Snow	United Kingdom	1.12.77
Dr. J. van Etten	Holland	31. 1.74

Chemistry and Biochemistry

Dr. A. Maradufu	Tanzania	1. 3.74
Dr. C. K. Wilkins	U.S.A.	25. 3.77

Sensory Physiology and Behaviour

Dr. J. V. Clark	United Kingdom	1. 1.77
Dr. J. H. MacFarlane	Canada	25. 4.77
Dr. R. A. Steinbrecht	Germany	1. 9.75–31.12.77

Insect and Animal Breeding

Dr. A. Basu	India	5.10.76

Insect Mass Rearing

Dr. R. S. Ochieng	Kenya	1.11.77
Dr. J. A. Odebiyi	Nigeria	10. 8.77–10.12.77

SCIENTIFIC OFFICERS

		Appointed
African Armyworm Research		
Mr. B. L. Otindo	Kenya	21. 1.75
Sorghum Shootfly Research		
Mr. K. Ogwaro	Uganda	1. 9.73
Termite Research		
Mr. B. M. Okot-kotber	Uganda	13. 2.76
Livestock Tick Research		
Mrs. C. K. A. Mango	Kenya	1. 7.71
Mr. D. K. Punyua	Kenya	1. 9.73
Tsetse Salivary Gland Physiology		
Mrs. N. Y. Patel	Kenya	1. 3.75
Mrs. M. Aruwa	Kenya	1. 2.75

Tsetse Reproductive Physiology			
	Mr. T. S. Dhadialla	Kenya	1.10.73
	Mr. J. Kawooya	Uganda	1. 9.73
Tsetse Acoustics			
	Mr. R. K. Saini	Kenya	2. 6.76
Histology and Fine Structure			
	Dr. E. D. Kokwaro	Kenya	1.12.75
	Mr. J. Owor	Uganda	1.12.73–1.10.77

RESEARCH ASSISTANTS

Livestock Tick Research			Appointed
	Mr. J. W. Chiera	Kenya	9.10.76
Tsetse Salivary Gland Physiology			
	Miss N. F. Darji	Kenya	1.10.74
	Mrs. J. A. Kongoro	Kenya	16. 4.74
Tsetse Reproductive Physiology			
	Mrs. R. W. Kunyiha	Kenya	18. 5.76

TECHNICAL STAFF

African Armyworm Research			Appointed
	Mr. J. Igunza	Kenya	6.11.72
	Mr. J. T. Kilori	Kenya	1.11.72
	Mr. M. Lubega	Uganda	1. 3.74
	Mr. D. N. Mathenge	Kenya	1. 6.73
	Mr. G. N. Mburu	Kenya	15. 1.74
	Mr. R. Okello	Kenya	1. 3.73
	Mr. C. Were	Kenya	1. 1.77
Sorghum Shootfly Research			
	Mr. G. M. N. Bizoza	Uganda	15. 9.77
	Mr. K. E. Kidega	Uganda	1.10.77
	Mr. J. C. Olela	Kenya	30. 9.77
	Mr. S. M. Othieno	Kenya	1. 4.73
Mosquito Research			
	Mr. E. Mkuzi	Kenya	1. 6.76
Termite Research			
	Mr. L. B. Bushairizi	Uganda	1.10.77
	Mrs. R. Kariuki	Kenya	1.10.74
	Mr. J. N. Kaseleweu	Kenya	1. 5.77
	Mr. D. T. Kasino	Kenya	1. 8.76
	Miss M. N. Mambea	Tanzania	1.10.74
	Mr. H. M. Nayeni	Kenya	1. 8.76
	Mr. J. O. Onyango	Kenya	12. 5.77
	Miss M. Wanjiru	Kenya	1.12.75
Livestock Tick Research			
	Mr. A. Bwire	Kenya	1. 9.75
	Mr. G. M. Hindi	Kenya	1. 3.74
	Mr. J. G. Mugane	Kenya	1. 8.73
	Mr. J. N. Ndungu	Kenya	1. 8.73
	Mr. R. Ojowa	Kenya	15. 2.72
	Mr. K. C. Wainaina	Kenya	1. 3.74

Tsetse Salivary Gland Physiology			
	Mr. A. A. Adema	Kenya	1.12.76
	Miss R. Chesang	Kenya	1. 3.72
	Mr. J. Likhanga	Kenya	1.11.74
	Mr. P. Onyango	Kenya	1.12.74
Tsetse Reproductive Physiology			
	Mr. F. Mukunza	Kenya	14.11.73
Tsetse Population Diversity			
	Mr. J. O. Apale	Kenya	1. 5.76
	Mr. F. Kathuli	Kenya	1. 1.77
	Mr. D. Uvyu	Kenya	1.12.74
Chemistry and Biochemistry			
	Mr. A. Chapya	Kenya	16. 4.74
	Mr. N. Juma	Kenya	1. 4.74
Histology and Fine Structure			
	Mr. M. Chimtawi	Tanzania	15. 1.74
	Mr. P. Lisamulla	Kenya	1. 2.73
Sensory Physiology			
	Mr. H. M. Kahoro	Kenya	1. 5.75
Insect and Animal Breeding			
	Mr. J. Atema	Kenya	1.10.75
	Mr. E. Awuoche	Kenya	1.12.73
	Mr. H. Banda	Kenya	16. 2.72
	Mr. A. Ikhunyalo	Kenya	16. 2.71
	Mr. J. Kagoiya	Kenya	1.10.73
	Mr. J. Ongudha	Kenya	1.10.73
	Mr. J. Wanyonje	Kenya	1. 2.70
Plant Resistance			
	Mr. S. H. O. Okech	Kenya	1. 8.77
Bioassay			
	Mr. G. Achieng	Kenya	1. 9.76
	Mr. L. Moreka	Kenya	1. 9.76
	Mr. B. N. Odero	Kenya	21.10.76
Workshop			
	Mr. H. Gichinga	Kenya	1. 3.73
	Mr. J. M. Maina	Kenya	1. 3.75
	Mr. J. N. Mtei	Tanzania	12.10.76
	Mr. P. O. Nyachieo	Kenya	1.12.73
	Mr. J. N. Omondi	Kenya	15. 7.74
	Mr. J. B. Omulo	Kenya	3. 9.73
Field Stations			
	Mr. S. Abdalla	Kenya	1. 6.76
	Mr. J. Mwandandu	Kenya	1. 1.77
	Mr. S. W. Wanjohi	Kenya	1. 6.76
Communications and Information			
	Mr. J. F. Shikhubari	Kenya	15. 8.75

SENIOR MANAGEMENT STAFF

Director	Professor T. R. Odhiambo
Manager for Communication Systems	Mr. J. M. Ojal
Administrative Manager	Mr. C. O. Angoma
Financial Manager	Mr. J. H. Jivanjee (resigned 31.5.77) Mr. L. Z. Mosha (appointed 17.10.77)
Accountant	Mr. R. M. P. Okura
Physical Plant Manager	Mr. M. L. Awuor (resigned 31.10.77)
Communications Officer	Mr. H. Awori (resigned 31.10.77)
Librarian	Mr. D. R. Kigera
Controller, Technical Services	Mr. Atashili Mando
Head, ICIPE Coastal Field Station	Dr. L. P. Lounibos (resigned 18.7.77)
Controller, Insectary Services	Dr. A. Basu
Senior Training Officer	Dr. L. O. Abe
Training Officer	Miss R. Washika

ADMINISTRATIVE STAFF

Miss E. Afandi	Copy Typist
Mrs. M. Antao	Assistant Secretary
Mrs. M. U. Arara	Senior Secretary
Mr. A. R. Ibrahim	Accounts' Clerk Trainee (resigned 7.11.77)
Mr. S. M. Kimaita	Administrative Officer
Mr. J. K. Kitur	Accounts' Clerk Trainee
Mr. M. P. Macohito	Store-keeper
Mr. B. Mwangi	Assistant Accountant
Miss D. A. Mbeche	Secretary
Miss L. O. Munge	Copy Typist/Clerk
Mr. A. Oguda	Assistant Accountant
Mr. W. O. Ogallo	Personal Assistant to the Director
Miss F. Ojode	Assistant Secretary
Mrs. R. A. Okoth	Copy Typist
Mrs. A. A. Okumali	Secretary
Mr. J. E. Okiri	Administrative Officer
Mrs. M. R. Opande	Secretary
Mrs. P. Owitti	Secretary
Mr. C. I. Rapasi	Purchasing Assistant
Miss M. Wafula	Assistant Secretary
Mrs. G. Weya	Telephonist/Receptionist

CONTENTS

SCIENTIFIC DEVELOPMENT: CHALLENGE FOR THE FUTURE

The year 1977 may well turn out in historical perspective for the International Centre of Insect Physiology and Ecology (ICIPE), to be the end of the childhood phase of the institute and the beginning of young adulthood. In that year, several remarkable developments took place. In the first place, the Government of Kenya, after several months of negotiations, concluded a new Agreement with ICIPE on 4th October 1977, which granted ICIPE full international status as an institute of advanced research in an area of direct relevance to the tropical world, and gave it a number of exemptions (including income-tax exemptions) and other benefits, to facilitate the work of this Centre. Undoubtedly, these facilities immediately created the environment necessary for creating confidence in the donors and users of ICIPE for its future and for its firm support by the host country, Kenya.

In the second place, an Inter-Agency Conference on the Development of ICIPE was convened in Nairobi on 26th and 27th July 1977, on a joint initiative by the Administrator of the United Nations Development Programme (UNDP) and the Executive Director of the United Nations Environment Programme (UNEP). This is the first time that the ICIPE donors—both current and potential—and other agencies and private foundations supporting ICIPE work, have come together to discuss ICIPE objectives, long-range programmes, training, and needs (both capital and operational). The conference accepted ICIPE strategy for research and training, and gave a strong recommendation that ICIPE be granted long-term and continuing funding, and that a donor framework be established in one way or another to give this recommendation a practical mechanism. The participants raised the question several times of more evident support of ICIPE by countries in Africa and other developing countries; and this matter is unquestionably one which will concern ICIPE management in the immediate future.

In the third place, ICIPE reached, in December 1977, an Agreement in principle, on cooperative research and training with the University of Ibadan, in Nigeria. Under the Agreement, to be signed early in 1978, ICIPE will receive graduate students from the University of Ibadan, who have already completed coursework requirements, to do their project work at ICIPE in areas of relevant importance to the Centre. The graduate students would then present their work as theses for higher degrees at Ibadan. This type of training has already been in operation in collaboration with the University of Nairobi; but it is the intention of ICIPE to make similar arrangements with leading university institutions elsewhere in Africa. The Agreement with Ibadan will represent the first foray in this endeavour.

In the fourth place, on a recommendation by the Governing Board of ICIPE, a new supporting and advisory body—the ICIPE Foundation—was established under Swedish law early in 1977 as a non-profit-making organization, specifically to support ICIPE (particularly in the intellectual field) and to strengthen ICIPE's communication with the international scientific community. The Foundation is a separate organization to ICIPE, and its secretariat is headquartered at the Royal Swedish Academy of Sciences in Stockholm. The Foundation held its first assembly in Oslo on 12th September 1977, at which time the forerunner of this body—the International Committee, which had been in existence for 7 years as an advisory committee of ICIPE—held its 8th and last meeting, and was dissolved. The Foundation membership consists of academies of science and similar scientific organizations throughout the world who have special expertise in insect science and who have a commitment to the ICIPE model for building up scientific capacity in the developing world to solve development-oriented problems. This young organization is an experiment in itself, in promoting intellectual communion with the young scientific communities and advanced research institutions in the developing world, in the latter's goal to solve their own priority science-based problems.

Finally, the ICIPE Governing Board underwent a major transformation in June 1977, during which time a revised Memorandum and Articles of Association for the Centre came into operation. From the beginning of ICIPE,

The Chairman of the African Committee (left) discusses a point with a research scientist in the Histology and Fine Structure Unit

members of the Board were elected on a personal basis; and because of the initial concern with the difficult business of institutional survival it was essential to establish beyond any doubt the scientific competence and excellence of ICIPE. The composition of the Board was therefore preponderantly one of senior scientists (both from Africa and elsewhere). With the firmer establishment of the Centre, it became increasingly evident that the management of the ICIPE enterprise has to become a major task of the Board. The new Board held its first meeting in September 1977; and its composition now reflects the realities of the future standing of ICIPE by having on its Board 16 members: 8 members elected by the ICIPE company from Africa and the rest of the world (senior personalities experienced in science policy, institutional management, and international donorship), 4 from the ICIPE Foundation, 3 from Kenya (representing the Ministries of Agriculture, Health, and Education), and the Director (as *ex-officio* member of the Board). It is expected that the Board will concentrate their deliberations on the long-range policy issues for this young institution.

The year 1977 saw the beginning of the process of consolidation of ICIPE programmes of research, which is likely to come to a peak by the end of 1980, when field station facilities for long-range ecological investigations would have been completed in three contrasting ecological situations.

The following reports on the progress of research work during the course of the year 1977 is a truly remarkable array of multidisciplinary advances in the target insect species that form the core of the ICIPE programme. If one were to make any selection, perhaps the two most advanced projects now are those on foraging termites and tsetse flies. The advances made here, both in the ecological and physiological facets, are a signpost to the future research thrusts of ICIPE as a whole.

Thomas R. Odhiambo
Director, ICIPE

1st March 1978.

TRAINING AT ICIPE, 1977

For long regarded simply as an appendage of ICIPE, training was formalized in 1977 and is now gaining tremendous momentum. The formalization saw the hiring of two training officers, Dr. L. O. Abe, Senior Training Officer and Miss Rose Washika, Training Officer. ICIPE, fully cognizant of its mandate to develop the scientific capacity and capability of Africa and other less-developed countries could no longer give training the low priority it was being accorded. The primary objective of the Training Department is to bridge the gap between the knowledge generated from research findings and that which is required for tackling problems related to pest management in tropical regions.

Research Associateships

Granted at the postdoctoral level to scientists already working in their own institutions, this programme saw its second candidate, Dr. J. Adebayo Odebiyi, Lecturer in the Department of Agricultural Biology, University of Ibadan, carry out ecological studies in Kenya on cowpea podborers from 10th August—16th December 1977. He also received some training in electron microscopy.

Graduate Training

Mr. John Kawooya, Associate Scientific Officer, completed his M.Sc. (University of Nairobi) on studies on the neuro-endocrine system of *Glossina morsitans* females during the second pregnancy cycle. He has now proceeded to the University of Illinois—(Urbana)—where he will continue his work on insect endocrinology for the next three years.

Mr. A. O. Mongi. From Tanzania, Mr. Mongi completed his M.Sc. thesis at the University of Nairobi on the water balance in *Rhipicephalus appendiculatus* and *R. pulchellus*. He has now registered for a Ph.D. at the same University to work on the feeding behaviour on immune and susceptible hosts of the ixodid tick *R. appendiculatus*. (Neuman, 1901).

Mr. Bernard L. Otindo. Mr. Otindo completed his two year study at the University of Nairobi. He is now preparing his M.Sc. thesis for submission. His work dealt with the morphology and histology of the sex pheromone gland in the African armyworm moth, *Spodoptera exempta* (Wlk.).

Miss M. R. Aruwa. A Scientific Officer in the Tsetse Programme, Miss Aruwa completed six and a half months research training in biochemistry and immunological techniques at the Institute of Microbiology, Universita Catolica Del Sacro Cuore, in Rome.

Mr. Jacob Yarro. From the University of Dar es Salaam, Tanzania, Mr. Yarro started training in May and continues his training in eco-genetics of the African armyworm (*Spodoptera exempta*), concentrating on the larval foodplant and phenotypical variation in these insects.

Mr. J. W. Chiera. A Research Assistant in the Tick Programme, Mr. Chiera attended a symposium on ticks in Neuchâtel, Switzerland, from 19th to 24th September 1977. The symposium, organized by the Institut de Zoologie, Université de Neuchâtel, dealt with biology and epidemiology of ticks with emphasis on *Rhipicephalus appendiculatus* and *Ornithodoros moubata*, the two most important species in ICIPE tick research.

The trainees sponsored by the United Nations Environment Programme (UNEP) joined ICIPE in July to undertake one year's research training. Mr. M. M. Ngunnzi, from the Division of Vector Borne Diseases (Ministry of Health, Kenya) is working on mosquito ecology with a view to setting up a reference index for *Aedes* in the African geographical region. Mrs. Theresa C. Aloo, from the Forestry Department (Ministry of Natural Resources) is working on termites as

Examination of termite mounds at Kajiado Research Station during the UNEP sponsored Group Training Programme, July 1977

cattle dung removers in semi-arid pasture. Mrs. Mary A. Owaga, Game and Wildlife Department (Ministry of Tourism and Wildlife) is working on the role of *Glossina longipennis* in the transmission of trypanosomiasis in a periodomestic situation.

Technical Training

(i) At the Polytechnic—Training of ICIPE technical staff continued at the Kenya Polytechnic in biological laboratory technology. Mr. Julius Apale, Miss Mercy Mambea and Mr. James Kagoiya participated in this programme.

(ii) In-house Training—An annual feature of ICIPE, the in-house training of technical staff in insect biology and biological techniques was conducted for a period of twenty-two weeks. This is aimed at increasing the efficiency of the technicians and improving skills in their day-to-day work.

Professional Training

Mr. John K. Kitur, Accounts Clerk, continued his correspondence course for Certified Public Accountants (CPA). Meanwhile, Mrs. Rose Opande, Secretary, enrolled with the French Cultural Centre for an advanced French course.

ICIPE Science Bursars Scheme

Aimed at motivating high-school science graduates, this scheme continued in 1977, engaging eight trainees. Their period of training was six months.

Other Training

Training in special techniques used in control of African animal trypanosomiasis was organized for two Somali technicians under the auspices of the Food and Agricultural Organization (FAO), Rome. Miss Khadra H. Warsame and Miss Zahra S. Elme received a six week training course in ecological skills: insect handling in a tsetse fly colony and salivary gland and reproductive physiology laboratory techniques.

Group Training Course

The first group training course on Components Essential for Ecologically Sound Pest Management Systems, co-sponsored by ICIPE and UNEP, was held at ICIPE, from 3rd to 22nd July 1977. The course was very successful and it has been decided that a similar course be held in 1978.

Last year, a total of 15 trainees representing Kenya (7), Malawi (2), Zambia (1), Ghana (3), India (1) and Somali (1) participated. This group of trainees comprised young scientists actively engaged in pest and vector management, as well as research scientists and university lecturers.

The course emphasized recent advances in the field of pest and vector management and environmental ecology; and a demonstration of the efficiency of new technologies in insect population management. To give practical in-depth appreciation of the ecological implications, the course was divided into lectures, case studies and field trips. Lectures included topics such as plant resistance as an approach to pest management, acquired immunity, traditional methods of pest management, relationships between pest intensity and yield losses, sampling techniques, cocoa pests, and new strategies for pest management. Practicals included the analysis and detection of population processes, susceptibility tests, key factor analysis, and data analysis. Case studies included: cocoa/pest relationships, the Lambwe Valley tsetse control project, malaria vectors, the WHO operational research programme on the control of riverine tsetse in West Africa. The field trips covered the ICIPE research station for termite study under semi-arid pasture conditions, the site for sampling tsetse populations and the domestic mosquito population study area. Lecturers for the course were drawn from university professors, WHO tsetse staff, ICIPE scientific staff and others from applied research institutes.

The recommendations stemming from the course stipulated the inclusion of more case studies dealing with animal/vector relationships—ticks and theileriosis, sandflies and disease and simulium and filariasis. In the area of plant/pest relationships, pests of cereals, grain legumes, roots and tubers, fibre crops and tree crops will be given attention. Appropriate field trips to complement lectures and case studies will also be arranged.

Livestock Tick Study Workshop

An annual feature of ICIPE, study workshops are organized on topical themes. This year the theme was "Physiological Significance of Tick Behaviour", held from 10th to 14th October and supported by USAID. It attracted many international research experts, including Dr. Rachel Galun, Head, Department of Entomology, Israel Institute for Biological Research and a Visiting Director of Research in the ICIPE Tick Research Programme; Professor A. Aeschlimann, Chairman of the workshop, from the Institute of Zoology, University of Neuchâtel, Switzerland and prominent scientists from Australia, Canada, Egypt, Southern Africa, Switzerland and the UK, the USA and West Germany. In all, 40 participants took part, 20 of whom came from institutions in Kenya, including ICIPE.

The Workshop examined critical areas useful in tick control work. These were: production of salivary gland antigen and water balance; feeding behaviour; environmental effects on diapause aestivation; sensory reception for host selection and pheromonal control of aggregation-attachment phenomenon. For future investigation, the workshop emphasized the integration of physiological and ecological studies to improve methods of control; the genetic manipulation of populations to effect control; host selection; the breeding of resistant cattle; studies of neuro-endocrine systems; the development of the use of natural acaricides as baits; and that methods of dissemination and exchange of information through training courses in tick biology and tick control be continued.

Participants in the Tick Study Workshop held at the Centre in October 1977

AFRICAN ARMYWORM RESEARCH

Visiting Director of Research
Professor J. W. S. Pringle (1970)

Honorary Project Leader
Dr. D. J. W. Rose (1977)

Research Staff
Mr. J. Igunza (1972) Technician
Dr. S. Khasimuddin (1973) Research Scientist
Mr. J. T. Kulai (1972) Technician
Mr. M. Lubega (1974) Technician
Mr. D. N. Mathenge (1973) Technician
Mr. G. N. Mburu (1974) Technician
Mr. R. Okello (1973) Technician
Mr. B. L. Otindo (1975) Scientific Officer
Dr. B. I. P. Persson (1977) Research Scientist
Mr. C. Were (1977) Technician

Collaborators
Dr. J. V. Clark, Sensory Physiology
Dr. M. H. den Boer, Research Scientist
Dr. R. A. Steinbrecht, Sensory Physiology

The armyworm research programme

D. J. W. Rose

Research at ICIPE during the last few years has made important contributions to the understanding of many aspects of the biology of the African armyworm. Some research has been in the form of well defined projects designed to answer particular questions in a short period of time. An example of this was the study made by Dr. den Boer of isoenzymes in populations of armyworm collected from widely separated parts of Africa. The results of this work, given later, provide support for the hypothesis that armyworm moth populations are highly mobile, and that control of this pest will only be achieved by close cooperation between neighbouring territories in East Africa.

Other investigations in progress are long-term, particularly those oriented towards understanding the relative importance of possible sources of moths which cause armyworm outbreaks. The questions which have to be answered are: Where do moths come from? How are moths concentrated in particular areas? What conditions favour outbreaks developing in these particular places? Where is the insect during the off-season?

An obvious source of moths is their emergence in large numbers from previous outbreak centres. Even so, less obvious sources may be just as important, and the investigations being made by Dr. S. Khasimuddin and Dr. B. Persson on the significance of long-term pupae and low density caterpillar populations are to be continued and intensified. As part of this, it is hoped that "The African Armyworm" will be adopted as a school ecological project and that this will enable populations of armyworm to be studied in detail in many different parts of Kenya. The active cooperation between ICIPE and the Kenya Science Teachers' College with initiation of a pilot field station is greatly welcome. Part of the school project will be monitoring populations of male armyworm moths by using simple pheromone traps. This has become possible now that the pheromone described by Beevor et al. (1975) has been developed and made available to ICIPE by the Tropical Products Institute, London.

The morphology of the female sex pheromone gland on the abdomen of the moth has been investigated by Mr. B. Otindo, and his findings are summarized later in this report.

Mr. J. Yarro's work has shown that armyworm fed on *Cynodon dactylon*, maize or *Pennisetum clandestinum* have lower mortality, higher growth rates and develop to larger caterpillars, pupae and moths, than do armyworms fed on the grasses *Setaria caudula* and *Panicum maximum*. He also suspects that mortality is least on *Cynodon dactylon*, which is the best known host of solitary-phase armyworm caterpillars in the field. The latter result is supported by Dr. Persson with his independent studies in field cages. Dr. Persson also found that caterpillars prefer *Cynodon dactylon* to maize.

Dr. J. V. Clark describes his studies on chemoreceptors on the mouthparts of armyworm caterpillar in the Annual Report of the Sensory Physiology Research Unit. His studies continue those started by Dr. Ma (ICIPE Annual Reports, 1974, 1975) and they are fundamental to understanding feeding by armyworm.

ICIPE's armyworm research is carried out in coordination with the Desert Locust Control Organization of East Africa; the Armyworm Forecast Unit, AFRO, Muguga; the Centre for Overseas Pest Research, London and Government entomologists of countries in East Africa who wish to develop a strategy for control of armyworm on a regional basis. Coordination of the work is the responsibility of the Project leader, Dr. D. J. W. Rose.

Reference

Beevor, P. S., Hall, D. R., Lester, R., Poppi, R. G., Read, J. S. and Nesbitt, B. F. (1975). Sex pheromones of the armyworm moth, *Spodoptera exempta* (Wlk.). Experientia **31**, 22.

Isoenzymes and the migration of the African armyworm, *Spodoptera exempta*

M. H. den Boer

Techniques for the separation of proteins have proved to be powerful tools in the study of genetic variation. Polymorphisms on protein levels can be used to study the structure of populations. In general, differences in allele frequencies can be found among populations in different parts of the distribution area of a species. If, however, enough gene flow occurs by migration, the whole system can be regarded as one panmictic unit and similar frequencies can be expected in the whole area.

African armyworms are caterpillars of the Noctuid moth *Spodoptera exempta*. They live on all sorts of graminaceous plants on which vast outbreaks can occur. Their economic importance can be considerable since they eat the main human food crops as well as pasture grasses. The occurrence of migration in *S. exempta* is known but its importance is a key controversial point. Outbreaks move during the year. These outbreaks could be caused by migrating animals or by increasing local populations when conditions are favourable.

The aim of this study has been to determine the relative importance of migration. Allele frequencies have been determined of six alleloenzymes that proved to be genetically polymorphic, an EST, β-HBDH, ODH, ∝⊥GPDH, ME and LDH. Seventeen armyworm samples have been collected at a maximal distance of 2000km in Kenya, Tanzania and Rhodesia on different food plants during 1975 and 1976.

No heterogeneity among these samples could be detected in the allele frequencies. A comparison with data from relevant literature on insects showed that the lack of heterogeneity cannot be ascribed to inadequacy of data. The occurrence of extensive migration it is concluded, causes the similarity in allele frequencies.

Some basic ecological studies on the African armyworm, *Spodoptera exempta*

S. Khasimuddin

It has been emphasized enough in the past that outbreaks of the African armyworm, *Spodoptera exempta* (Wlk.) are extremely sporadic throughout its distribution range. The virtually complete disappearance of the insect for several months in a given year is also well known. However, the causes for such disappearance have not yet been investigated. It therefore becomes essential to investigate into the survival strategy of the insect in order to gain a better understanding of its ecology as a whole.

There are various possible explanations that can be given for the disappearance of the insect during unfavourable periods of the year. The following two possibilities are under detailed investigation:

(1) The insect passes the unfavourable period by some form of adaptation—diapause?

(2) The insect survives in specialized niches, but at density levels that are too low to be noticed.

In its range of distribution the insect may be behaving in either one or the other way or even a combination of the two possibilities at various places. Investigation into both possibilities has been initiated and is briefly discussed below.

Field-cage studies

In order to check whether the insect is capable of breeding throughout the year in Nairobi, studies were initiated in a field-cage where ample food was provided for all life history stages.

The scope of this report does not allow the inclusion of all the detailed data. Such data has been summarized in Tables 1 and 2 to bring out the major points of interest. Table 1 shows the time taken by complete generation (egg-adult) and the time taken in the pupal stage. It can be seen that the total generation time varies from about 30 days to 79 days. In great part this variation seems to be due to the varying pupal durations (1 week to 8 weeks), the development of the larval instars being more or less regular throughout the year. It must be observed that the longest periods spent by any generation happen to be during the cold and dry months of the year. The overall survivorship varies between 1% to 10%, but can be

Table 1. Summary of generation times of *Spodoptera exempta* reared throughout the year in field cages at Nairobi

	Jan./Feb.	Feb./Mar.	Mar./April	Apr./May	May/July	Sept./Nov.	Nov./Dec.
Total duration (egg-adult)	44 days	51 days	44 days	>30 days	71 days	79 days	58 days
Pupal duration (approximate)	4 weeks	2 weeks	1 week	?	8 weeks	8 weeks	5 weeks
Overall survivorship	1.0%	2.0%	3.0%	0	4.0%	3.0%	10.0%

considered to average around 3.3%, meaning that only 3–4 adults accrue from every 100 larvae hatched from eggs. The results confirm earlier findings that the insect is capable of breeding throughout the year in many places provided food supply is good. (Hattingh 1941, E. Brown, unpublished manuscript.)

Table 2 summarizes the data on survivorship and mortality during various life-history stages. It may be observed that high mortality occurs during the pupal stage. Examination of the causes of mortality revealed that this high pupal mortality is caused to a great extent by a suspected "Cytoplasmic Polyhedrosis virus (C.P.V.)", which kills the imago in the puparium before adult emergence. Such a situation is of common occurrence in field populations also and has been reported earlier (ICIPE Annual Report, 1975). It may also be seen that the first 3 larval instars also show a considerable mortality, although the extent of such mortality varies during different times of the year. No definite causes of such mortality can be given at present as observations on these three stages have been very difficult due to the minute size of the larvae.

Studies on diapause

It has been reported earlier (ICIPE Annual Report, 1975) that pupae from larvae collected at various places from outbreaks exhibit a phenomenon of delayed emergence, (diapause?) passing as long as six months in the pupal stage. Diapausing individuals exhibit a marked reduction in their oxygen uptake (ICIPE Annual Report, 1976). Among other aspects, the measurement of oxygen consumed by pupae can serve to identify pupae exhibiting such a phenomenon. Measurements of oxygen consumption were therefore carried out on pupae resulting from collections at various sites during the outbreak season. The apparatus used was "Shoelander's microvolumetric respirometer" which is a modification of the conventional warburg apparatus. Oxygen consumption by pupae from the laboratory colony (Figure 1) served as the standard.

Results are presented in Figures 1, 2 and 3. Figure 1 (standard) suggests that as soon as the pre-pupal larvae pupate the oxygen consumption is high. This consumption drops after the 2nd day and then increases considerably 48 to 72 hours before eclosion. Data from two of the many sites is presented in Figures 2 and 3, showing a similar general pattern. However, it is noteworthy that the period of development is prolonged and that the oxygen consumption increases quite considerably before eclosion, which is different from the pattern exhibited by the standard laboratory reared pupae. It is also worth mentioning that at neither of these two

Table 2. Survivorship and mortality of *Spodoptera exempta* at different life-history stages during the year based on field cage studies

	Jan./February		February/March		March/April		April/May		May/July		Sept./November		Nov./December	
Life-history Stage	Lx	%	Lx	%	Lx	%	Lx	%	Lx	%	Lx	%	Lx	%
1st instar	1.00	29.0	1.00	72.0	1.00	71.0	1.00	9.0	1.00	—	1.00	35.0	1.00	—
2nd instar	0.71	64.7	0.28	10.7	0.29	17.2	0.91	32.9	—	—	0.65	30.0	—	23.0
3rd instar	0.25	32.0	0.25	52.0	0.24	16.6	0.61	29.5	—	34.0	0.45	11.1	0.77	38.9
4th instar	0.17	41.1	0.12	41.6	0.10	20.0	0.43	27.9	0.66	16.6	0.40	15.0	0.47	23.9
5th instar	0.10	50.0	0.07	28.5	0.08	25.0	0.31	58.0	0.55	10.9	0.34	20.5	0.36	36.1
6th instar	0.05	20.0	0.05	—	0.06	16.6	0.13	—	0.49	10.2	0.27	25.1	0.23	—
Pre-pupa	0.04	—	0.05	20.0	0.05	20.0	—	84.0	0.44	18.1	0.20	—	—	30.4
Pupa*	0.04	75.0	0.04	50.0	0.04	25.0	0.02	100.0	0.36	88.8	0.20	85.0	0.16	37.5
Adult	0.01		0.02		0.03		—		0.04		0.03		0.10	

Lx = Survivors in stage x/initiated cohort
% = Percent mortality from the previous stage
* = High mortality during pupal stage to be noted

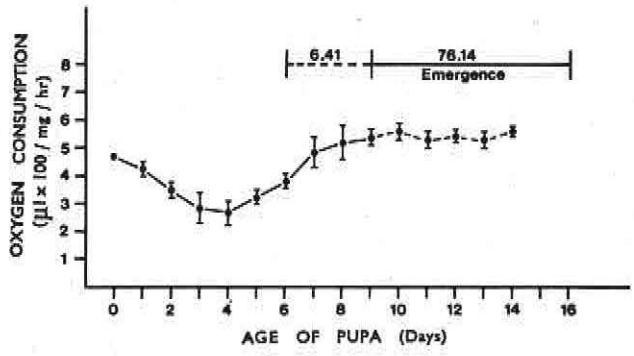

Figure 1. Oxygen consumption by pupae at various ages—insectary stock "standard"

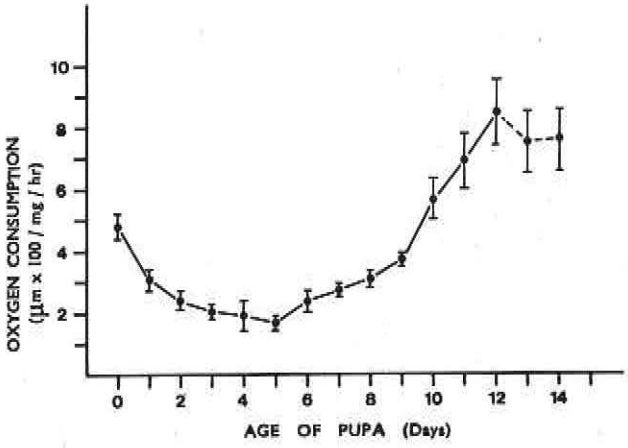

Figure 2. Oxygen consumption by pupae at various ages—pupae from Isiolo

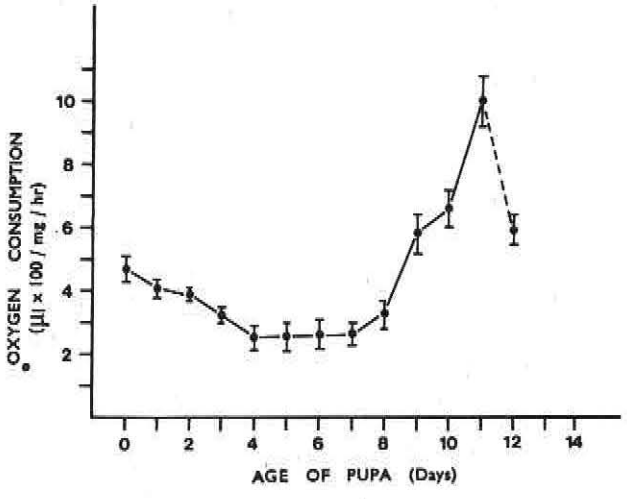

Figure 3. Oxygen consumption by pupae at various ages—pupae from Nanyuki

places, nor at any other of the many more sites of collection, did a single pupa of the several thousand tested, show any signs of delayed development. This however, does not preclude the possibility of occurrence of a "diapause".

Detailed investigations on various other aspects have been initiated but are rather premature to report here.

Reference

Hattingh, C. C. (1941). The biology and ecology of the armyworm (*Laphygma exempta*) and its control in South Africa. Sci. Bull. Dept. S. Africa. **217** 50 pp.

Low-density populations of *Spodoptera exempta*

B. I. P. Persson

Preparations have been made and experiments initiated for a long-term study of the significance of low density populations of the African armyworm as a source of moths which cause armyworm outbreaks.

Essentially these preparations include:

(1) Determining locations in Kenya where conditions are potentially suitable for breeding for all or part of the year.

(2) Developing effective survey methods.

(3) Making ecological studies of low-density populations in field locations.

Preparations which have thus far been made include:

(1) Building of a large ($6 \times 6 \times 3$m) plastic netting cage for outdoor rearing and observations on *Spodoptera exempta*.

(2) Building of an automatic mercury light trap separating the catches before and after midnight and recording the wind direction.

(3) Designing and testing of a simple field research station to be used in a nationwide monitoring net for the African armyworm. The stations will serve as a compliment to the existing AFRO monitoring net and will have the following activities:

(a) Monitoring of adult populations by means of a mercury light trap and pheromone traps.

(b) Continuous rearing of *S. exempta* in an outdoor rearing cage in order to try to establish whether or not the local climatic conditions allow the armyworm to survive in the area all year round. This work will also give indications as to the possible number of generations and the developmental time for each generation.

(c) Field surveys of pastures and crops in order to try to establish whether or not local populations exist.

The emphasis will be on detection of low density populations (if such exist) during that part of the year when no outbreaks are reported.

This will demand the establishment of a network of field stations where population studies are conducted. We hope that the field stations will be run by high school students as part of their biological training. The final design of the stations will be done in cooperation with

pedagogical experts at the Kenya Science Teachers' College. Strong efforts have been made to properly cover the training aspects of the project. A pilot station was established at the KSTC in the middle of January 1978. The network is intended to be permanent and can be extended in accordance with available funds.

The larval food plant and phenotypical variation in the African armyworm, *Spodoptera exempta*

J. Yarro

The larvae of the African armyworm moth, *Spodoptera exempta* feed on plants belonging to the families Gramineae and Cyperaceae. They consume large quantities of food during the fifth and sixth instar stages and thus destroy one plant after another in an area of high larval density. This means that in a natural habitat where different grass species co-exist the larva has the chance to feed on a number of grass species during its life time.

The purpose of this research project is to work out differences between populations of armyworm moths reared on different grass species.

In order to do this, five species belonging to the grass family, Gramineae, namely *Zea mays*, *Cynodon dactylon*, *Pennisetum clandestinum*, *Panicum maximum* and *Setaria caudula* were used as food plants. The larvae were kept on the same food plant species throughout the larval life time. The grass leaves were thoroughly washed with water before introducing them to the larvae. The larvae were kept in kilner jars in the insectary at a temperature of 25°C and 70% R.H. Except for the studies on larval mortality the larvae were kept at the density of not more than ten per one pound kilner jar and not more than twenty per two pound kilner jar.

Larval mortality

The larval mortality was studied by introducing 125 newly hatched first instar larvae on each of the five plants. The number of larvae dying in each instar was recorded.

The larval mortality distribution showed that the five plants used as larval food plants could be broadly divided into two groups ie. *Z. mays—C. dactylon—P. clandestinum* group and *P. maximum—S. caudula* group. In the former group the mortality distribution is U-shaped, the highest mortality rate occurring in the first two and the last two instars.

On the basis of survival up to the adult stage *C. dactylon* is the best grass as larval food. *P. maximum*

Table 1. Larval and pupal duration, sex and larval food plant recordings

Food Plant (Grass species)		Larval duration		Pupal duration	
		n	mean ± S.E.	n	mean ± S.E.
Z. mays	Males	72	13.6±0.1	72	9.7±0.1
	Females	61	13.7±0.2	61	9.7±0.2
C. dactylon	Males	36	13.6±0.6	36	10.5±0.1
	Females	37	13.6±0.2	37	10.0±0.2
P. clandestinum	Males	21	15.4±0.4	21	10.7±0.3
	Females	22	15.0±0.8	22	10.5±0.2
P. maximum	Males	9	19.0±0.8	9	10.4±0.3
	Females	5	18.0±1.5	5	10.8±0.6
S. caudula	Males	13	19.0±0.9	13	9.5±0.3
	Females		19.0+1.0	17	9.4±0.2

Table 2. Pupal and adult weights

Food Plant (Grass species)		Pupal weight (mgm)		Adult weight (mgm)	
		n	mean ± S.E.	n	mean ± S.E.
Z. mays	Males	72	182.2±2.99	67	86.6±1.77
	Females	60	184.3±3.05	56	96.0±1.86
C. dactylon	Males	33	186.2±3.50	35	83.0±1.89
	Females	36	187.8±2.92	36	94.8±2.52
P. clandestinum	Males	21	183.1±5.85	20	77.8±3.19
	Females	22	190.5±6.01	21	88.7±3.94
P. maximum	Males	9	183.6±5.87	9	78.2±3.12
	Females	4	153.6±11.16	4	73.0±8.29
S. caudula	Males	13	142.0±7.08	13	63.6±2.70
	Females	17	144.5±5.22	17	66.7±3.32

and *S. caudula* are the poorest of the five grass species used as larval food plant. *Z. mays* and *P. clandestinum* fall in between *C. dactylon* on one side and *P. maximum* and *S. caudula* on the other.

Development

The larval and pupal duration, sex and the larval food plant were recorded for each individual. The results of duration in days are presented in Table 1.

The results show that larval growth is slowest if the larvae are reared on *P. maximum* and *S. caudula* but fastest when they are reared on *Z. mays* and *C. dactylon*. The growth rate of the larvae reared on *P. clandestinum* comes in between.

Pupal and Adult Weights

Pupal and adult weights were taken within twelve hours of pupation and emergence respectively. In the case of the adults weights were taken after the meconium had been voided. The results are shown in Table 2.

The results show that pupae and adults reared on *Z. mays*, *C. dactylon*, *P. clandestinum*, and male pupae and adults reared on *P. maximum* weigh more than individuals reared on *S. caudula* and females reared on *P. maximum*. In addition females weigh slightly more than males except in the sample reared on *P. maximum* where the females weigh less than the males. The greater female weight is probably due to the weight of eggs. The discrepancy in the sample reared on *P. maximum* cannot be explained until the sample size is increased.

Wing length measurements

Both fore and hind wing lengths were measured using a vernier steel rule. In each case the distance between the wing base and outer edge of the tip of the wing was measured. Estimation of the lengths of damaged wings was avoided as this could introduce an error. The results are shown in Table 3.

Both fore and hind wings in individuals reared on *Z. mays*, *C. dactylon* and *P. clandestinum* and in males reared on *P. maximum* are slightly longer than they are in individuals reared on *S. caudula* and in females reared on *P. maximum*. Females tend to have longer fore and hind wings than the males except in the sample reared on *S. caudula* where the males have slightly longer fore wings than the females.

Wing areas

The wing areas were estimated using a graph paper method (Table 4). An intact wing was placed on graph paper and a line drawn along its edges. The number of squares enclosed by the line was then counted to give

Table 3. Wing lengths

Food Plant (Grass species)		Fore wing length(mm)		Hind wing length(mm)	
		n	mean + S.E.	n	mean + S.E.
Z.mays	Males	58	14.8±.09	57	11.3±.08
	Females	54	15.6±.10	53	11.8±.07
C.dactylon	Males	31	14.7±.15	31	11.3±.13
	Females	33	15.6±.15	33	11.9±.14
P.clandestinum	Males	17	14.3±.27	17	11.2±.21
	Females	21	15.6±.17	21	11.9±.11
P.maximum	Males	7	14.8±.25	7	11.3±.21
	Females	3	13.7±.75	3	10.6±.68
S.caudula	Males	12	13.9±.19	12	10.6±.14
	Females	17	13.7±.34	16	10.8±.21

Table 4. Wing areas

Food Plant (Grass Species)		Fore wing areas (mm^2)		Hind wing areas (mm^2)		Total Wing areas (mm^2)	
		n	mean ± S.E.	n	mean ± S.E.	n	mean ± S.E.
Z.mays	Males	58	130.1±2.34	58	9.3± 1.99	59	231.0± 3.52
	Females	53	137.5±2.49	53	114.1± 3.63	53	251.5± 5.22
C.dactylon	Males	31	130.7±2.82	31	111.5± 4.87	31	242.0± 6.64
	Females	33	136.1±3.40	33	106.5± 4.08	33	243.0± 5.28
P.dandestinum	Males	18	124.0±4.52	18	96.9± 4.99	18	226.5± 7.20
	Females	21	139.4±3.63	21	110.2± 4.91	21	249.6± 7.22
P.maximum	Males	7	131.3±4.46	7	93.1± 6.27	7	224.5± 7.99
	Females	3	117.0±6.00	3	107.3±19.06	3	224.3±24.48
S.caudula	Males	12	112.1±4.15	12	87.8± 4.29	12	199.1± 7.36
	Females	16	124.5±6.05	16	94.2± 6.37	17	210.8±13.16

the estimate of the wing area. Fractions of squares below 0.25mm² were not counted and those above 0.25mm² and below 0.50mm² were rounded to 0.50mm².

The fore wing areas in females reared on *P. clandestinum*, males reared on *P. maximum* and in individuals reared on *Z. mays* and *C. dactylon* are larger than the fore wing areas in females reared on *P. maximum*, males reared on *P. clandestinum* and in individuals reared on *S. caudula*. There is less variation in the hind wing areas but the males reared on *P. clandestinum*, *P. maximum* and *S. caudula* seem to have the least areas. The total wing areas are smallest in males reared on *P. clandestinum* and in individuals reared on *P. maximum* and *S. caudula*. The wing areas tend to be larger in females than in males except the fore wings of individuals reared on *P. maximum* and the hind wings of individuals reared on *C. dactylon*. The total wing areas are almost equal in both sexes of individuals reared on *C. dactylon*.

Longevity

Each adult was kept individually in a kilner jar and was fed on 10% sucrose solution. The longevity of each individual was recorded and the results are shown in Table 5.

Table 5. Longevity recordings

Food Plant (Grass Species)		Longevity (days)	n
Z. mays	Males	9.40±.53	55
	Females	7.82±.32	50
C. dectylon	Males	9.23±.55	31
	Females	8.60±.69	30
P. clandestinum	Males	9.57±.99	17
	Females	9.80±.64	20
P. maximum	Males	12.00±1.64	5
	Females	8.6±1.20	3
S. caudula	Males	12.42±1.49	12
	Females	9.33±91	15

The results show that, generally, males live longer than females except in the sample reared on *P. clandestinum* where the females live slightly longer than the males. More significant is the observation that males reared on *P. maximum* and *S. caudula* live longer than males reared on *Z. mays*, *C. dactylon* and *P. clandestinum*.

These conclusions have been based on very small samples of some of the food plants, particularly *P. maximum*, but samples reared on *S. caudula* and *P. clandestinum* also need to be increased in order to obtain conclusive results.

Outdoor cultures of armyworm on maize and *Cynodon dactylon*

B. I. P. Persson

Outdoor cultures of the armyworm were started in the large rearing cage in October 1977. So far, two generations have been completed. The larvae are being reared on maize and stargrass (*Cynodon dactylon*). In the first generation 1% of the larvae (4 out of 400) resulted in adults in the maize cultures against 9% (36 out of 400) in the cultures reared on stargrass. The larvae grew quicker on maize but the mortality was very high because of virus diseases, particularly in the last instars. The second generation emerging at the end of December 1977 produced a very low relative number of adults as mortality was extremely high among the first and second instar larvae during the heavy rains occurring in the beginning of the larval period. Only three adults emerged. Rearing attempts will be made during all of 1978 and the cultures will be extended.

Food preference tests were conducted in the form of a dual choice experiment. Four hundred larvae were evenly distributed on six maize plants and six stargrass plants arranged in such a way that the larvae could freely move from one plant to the other. Three counts were made in the first generation:

Count 1 (third and fourth instar larvae) 29 larvae were found on grass and 8 on maize.

Count 2 (fourth and fifth instar larvae) 19 larvae on grass and 4 on maize.

Count 3 (fifth and sixth instar larvae) 16 on grass and 4 on maize.

Thus there was a significant preference for *C. dactylon*. It seems that the larvae can choose the host plant that gives the highest survival rate.

Morphology and histology of the female sex pheromone gland in the armyworm moth, *Spodoptera exempta*

B. L. Otindo

A study was made of the structure of the pheromone gland in female armyworm moths of the genus *Spodoptera exempta* (Wlk). Electroantennogram bioassays showed that the gland in this moth, as in other moths, is a modified intersegmental membrane between segments eight and nine. It is a ventral sac measuring from 1.0 to 1.3mm when the abdomen is fully protruded.

Gland cells are larger and columnar compared to the small and squamous cells in the unmodified intersegmental membranes. They measure 5.8µm high in pharate individuals. Height increases in postemergence age until 4 days when there is no further significant increase. Maximum gland height is about 12µm. The gland surface structure is characterized by extensive

folding and cuticular eversions, mammiform and tapering in shape, which bear pores at their tips.

Transmission electron microscope studies provided additional information on gland cells and the overlying cuticle. The pores seen in cuticular eversions are formed by the epicuticular filaments which extend through the dense epicuticle and end up to the less electron dense cuticulin layer. Epidermal vacuoles, on the other hand, are evident as lipid droplets, morphologically continuous with the granular cytoplasm, and membrane bound vesicles. They are closely associated with granular (rough) endoplasmic reticulum. The cytoplasm furthermore contains a few microbodies, located near the nucleus, and numerous mitochondria. The plasma membrane is modified into basal and lateral involutions and apical microvillae.

It is suggested from morphological evidence that the gland cell is active and microcirculation of pheromone or the precursor material is facilitated by the modified membranes.

SORGHUM SHOOTFLY RESEARCH

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Population dynamics of *Atherigona soccata* in the field

J. R. Clearwater and S. M. Othieno

The purpose of the studies carried out during 1977 was to replicate the 1976 investigation of the population dynamics of *Atherigona soccata* populations in the field.

Methods used were essentially the same. The Indian variety CSH-1 was used as the susceptible variety replacing an indeterminate local variety. Counting of dead hearts in a random sample of a hundred replaced dissection of the infected plant and counting of the individual larva. Four replicates of CSH-1, two of Serena and one of the indeterminate variety were planted.

Excellent rainfall in 1977 appeared to contribute to a marked increase in populations of *A. soccata*.

Eggs are laid more frequently on CSH-1 (2/plant) compared with Serena (0.5/plant) (Figure 1). The results are very similar to those obtained in 1976. The 1976 conclusion that a significant component of resistance in Serena is due to a lower number of eggs laid appears to be valid. Standard deviations of height over the period of growth show that susceptible size classes are present in both varieties throughout the year. Thus a different growth strategy does not explain the lower numbers of eggs on Serena.

The numbers of dead hearts is a measure of successful larval establishment. Distinct central shoot wilting revealed the presence of second or younger instars. Dry, brown, dead hearts indicated the presence of an early third instar. The later stage was scored. In both varieties one larvae establishes itself for every 2–3 eggs laid (Figure 2).

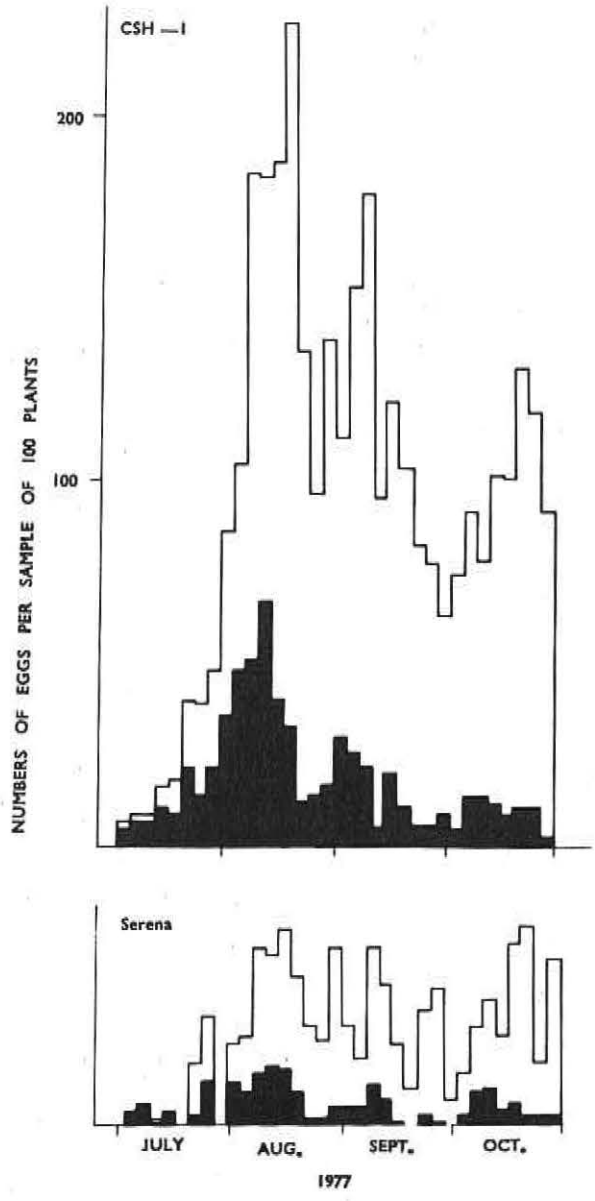

Figure 1. Oviposition records for CSH-1 and Serena
□ hatched eggs
■ freshly laid eggs

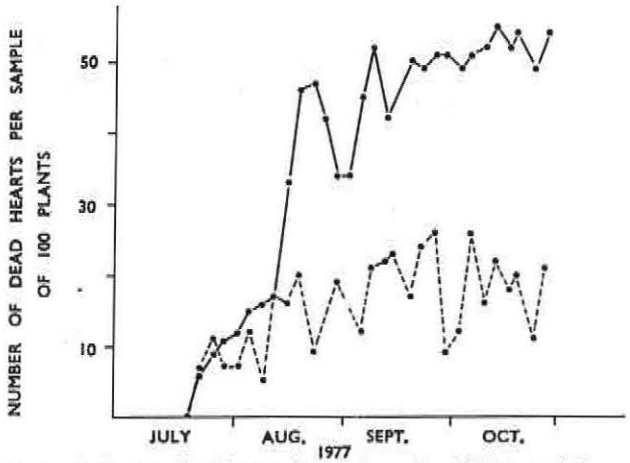

Figure 2. Brown dead hearts in sorghum for CSH-1 and Serena
●—● CSH-1
●---● Serena

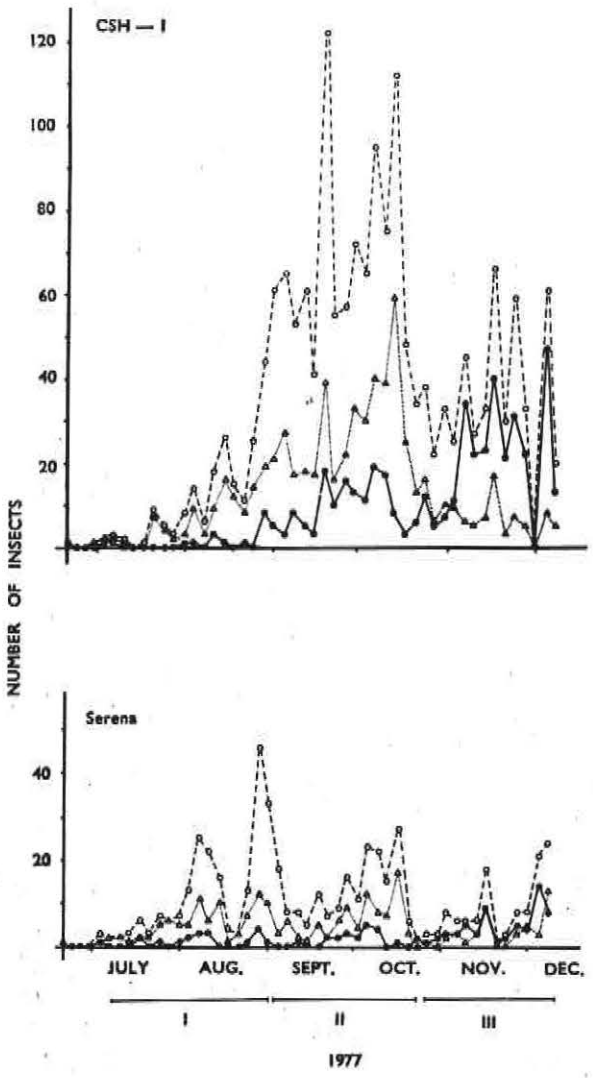

Figure 3. Captures of females with baited trap on CSH-1 and Serena
●—● Nulliparous females
△.....△ Multiparous females
o---o Total females
I Invasion
II Population build-up
III Rain induced synchronous emergence of adults

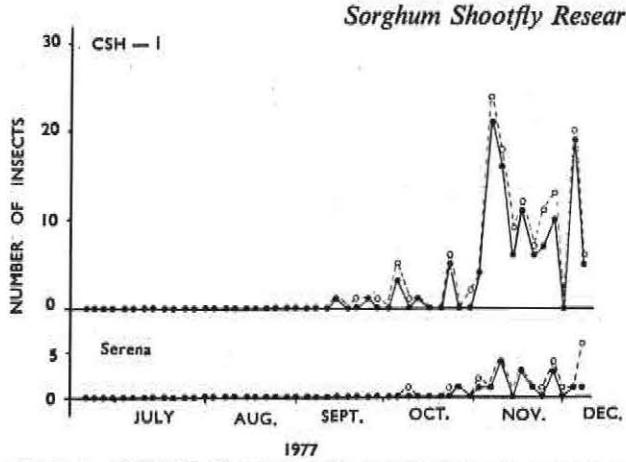

Figure 4. Captures of males with baited trap on CSH-1 and Serena
●—● Freshly emerged males
o--o Total males

Adult *A. soccata* found above a sorghum crop belong to one of three phases: I Invasion; II Population build-up; III Rain induced synchronous emergence of adults (Figure 3).

In August and early September, equal numbers of adults can be found over CSH-1 and Serena. Reference to Figure 2 shows that dead hearts are increasing rapidly in mid-August. As it takes a further three weeks to generate adults (one week for completion of the third instar plus two weeks for pupation) the crop under consideration will not be producing many adults till early September. It is possible that these early peaks represent an accumulation of individuals from the previous crop. This is the only time that equal populations are observed. Usually the CSH-1 crop has a four to five times greater population, an apparent consequence of the initial difference in the level of egg laying.

A population build-up on both crops occurs in September and October. As observed in 1976, multiparous females accumulate and form the dominant age class at the end of the season.

Towards the end of the season, unattacked plants begin to form seed heads. Formation of the panicle primordium precludes use of the apical meristem by the larval shootfly. In both varieties, live larvae were not observed later than the 4–5mm primordia stage. The adult population also suffers reverses when the short rains begin. The sharp drop in multiparous females during the November rains suggests heavy mortality and/or immigration. Two weeks after the onset of the rains the population again rises. Newly emerged adults make up the bulk of the population (70–80%). This is the only time that males, also freshly emerged, are caught in the baited trays (Figure 4).

During the 1977 dry season, there were two short heavy showers (grass rains). Two weeks later, the very low, dry season population increased with the addition of newly emerged females and males. The numbers were modest, but were shown in baited traps, sweep and suction traps.

In 1976 heavy rain caused a big drop in the numbers of larvae in the dry stems of the previous crop of sorghum. Several larvae were caught in the process of moving from the plant into the soil for population.

Whenever a previous crop was removed to make way for a new planting or ratoon, small numbers of larvae were always found. Two-and-a-half years of careful trapping and dissection on local grasses failed to uncover a dry season population of *A. soccata*. Only during the height of the population build-up on sorghum, could small numbers of *A. soccata* be found on the local grasses. Preliminary measurements revealed third instar larvae with a depressed rate of respiration. This quiescence was easily terminated. Larvae from dry season crops rapidly pupated when placed on a moist diet.

It is concluded that *A. soccata* moves over the dry season as quiescent III larvae in remnants of the old crop. In India, the root bases are left in the field following the harvesting of the stems for fodder. Rain causes the larvae to move into the soil for pupation. The number of larvae moving is roughly proportional to the amount of rain fallen.

The populations of *A. conigera*, *A. trapezia* and *A. laevigata* varied in the manner reported for 1976.

The following species list for Nairobi has been compiled:

(1) *Acritochaeta orientalis*
(2) *A. yorki*
(3) *Atherigona soccata*
(4) *A. laevigata*
(5) *A. trapezia*
(6) *A. conigera*
(7) *A. hancocki*
(8) *A. gilvifolia*
(9) *A. mirabilis*
(10) *A. naqvii*
(11) *A. longifolia*
(12) *A. lineata* ssp. A
(13) *A. lineata* ssp. B
(14) *A. steelae*
(15) *A. secrecauda*
(16) *A. matelei*
(17) *A. tetrastigma*
(18) *A. cinerina*
(19) *A. albistyla*
(20) *A. ruficornis*
(21) *A. binubila*

Four new species have been found. Numbers 3, 4, 6, 11, 12, 19, 20 and *A. tomentigera* have been identified from samples submitted by K. Ogwaro. This should be regarded as an incomplete list. Seasonal variations in population of the above species were recorded.

Work on the sexual behaviour and trifoliate organ ultrastructure continued in 1977.

Editor's note. The author would have preferred to see Figures 3 and 4 reproduced to twice the size shown.

Observations on longevity and fecundity of the sorghum shootfly, *Atherigona soccata* Rond. (Diptera, Anthomyiidae)

K. Ogwaro

Longevity and fecundity of the sorghum shootfly, *Atherigona soccata* Rond. were mainly affected by adult food. Lack of mating reduced fecundity and affected the rate of oviposition in the females, but did not affect female longevity. The mean number of eggs per mated female was 62.8 ± 0.9 (range 10–167). Oviposition began at a mean period of 4.7 (range 2–14) days after adult emergence. Mean longevity of females and males was 32.6 ± 0.3 and 25.4 ± 0.4 days respectively.

The sorghum shootfly, *A. soccata* is the most damaging insect to sorghum in East Africa. Continuous attacks on newly sown sorghum result in either complete destruction of the crop or in drastically distorted plants. Little is known yet about the biology of this insect although some studies have been made in India and in East Africa. Studies were, therefore, conducted during 1975 and 1976 at Kibos, a typical sorghum growing area of the East African region, to elucidate certain aspects of shootfly biology.

All observations were made in the greenhouse at the Cotton Research Station of the Ministry of Agriculture, Kenya. Sorghum of the variety Serena was used in all experiments. Serena is currently the recommended sorghum for lowland areas (below 5,000 ft, 1,530 metres) of East Africa. It shows appreciable levels of resistance to *A. soccata*, witchweed (Striga), and leaf diseases. Laboratory reared *A. soccata* were used throughout.

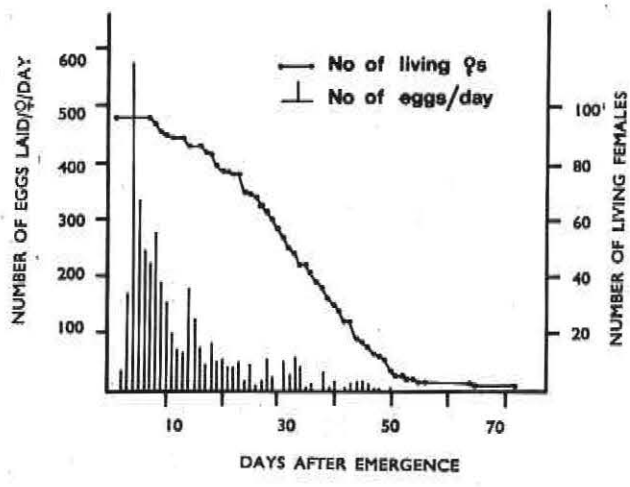

Figure 1. Longevity and rate of oviposition by 96 females depositing in separate cages

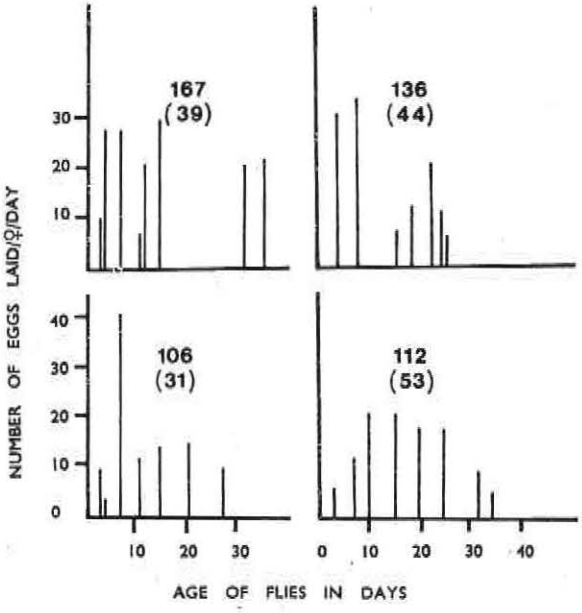

Figure 2. The normal pattern of oviposition as represented by 4 females depositing in separate cages. The figures within parenthesis are longevity in days

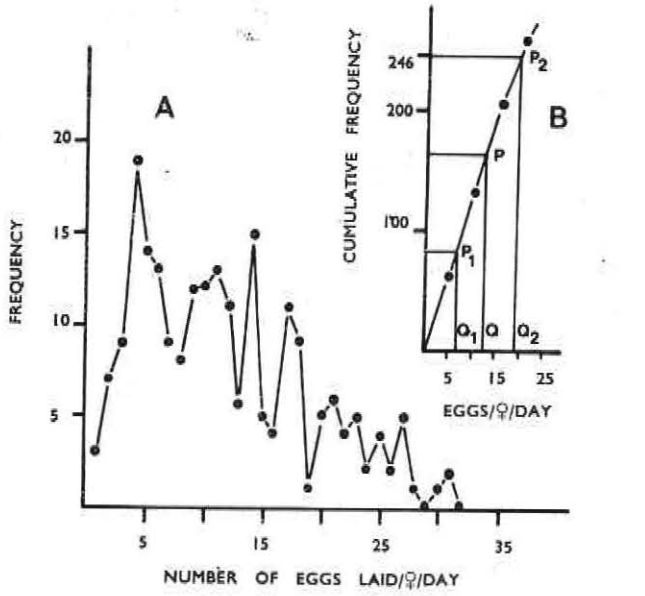

Figure 3. Frequency distribution of eggs per female per day
(A) in cumulative frequency
(B) showing a normal range of more than 5 and less than 20 eggs per female per day with a mode of 13.8

Rearing procedure

The greenhouse population was started by introducing field-collected males and females in plastic oviposition cages measuring 23×11×11cm, which contained sorghum plants of from 3 to 7 leaf stages. When eggs were laid the plants were kept, and 4 or 5 days afterwards the dead heart symptoms appeared. They were dissected and the larvae transferred into new plants. The stems were cut and, using forceps, the leaf sheath was opened enough to allow easy penetration by the larva. The larvae were checked regularly for pupation, and when necessary, the plants were changed. The pupae were transferred into specimen tubes containing moistened filter papers. When the adults emerged they were transferred into the oviposition cages, where fecundity and longevity were studied.

Egg numbers and longevity

The adult longevity and the number of eggs laid during the entire adult life were recorded in the oviposition cages placed in the greenhouse. The laboratory reared male and female flies were put into the cages containing sorghum plants. The flies were maintained on brewer's yeast, glucose and water. To test the effect of mating on longevity and fecundity, the females were placed in the oviposition cages without males, immediately after their emergence. The effect of adult food was tested by maintaining one set of flies on brewer's yeast, glucose and water and the other set on glucose and water alone. The host plant effect on fecundity and longevity was tested by comparing the fecundity and longevity of flies which were enclosed on sorghum throughout, to those which had each of the test plants at intervals of 5 days. The five test plants were presented in the order *Sorghum*, *Digitaria scallarum*, *Rottboellia exaltata*, *Setaria verticillata* and *Panicum maximum*, depending on the order of their emergence; ie. if the first emerging female was presented with sorghum first, the second emerging female would be given *Digitaria* first. A total of 47 females were involved, all of them being presented with each of the test hosts at least once, since the minimum life span was 27 days.

Fecundity

The total number of eggs recorded in the greenhouse was 6,029, laid by 96 mated females. The mean number of eggs per mated female was 62.8±0.9 (range 10–167). The results of a series of experiments on fecundity are summarized in Table 1. Two important parameters can be obtained from these results, viz: the mean total number of eggs deposited per female and the mean number of eggs deposited per female per day. The results show that, under the conditions of the experiments (21.8±0.8°C. and 62.1±7.4 R.H.%), *A. soccata* deposited a mean number of between 61 and 65 eggs per female with a maximum of 167 and a minimum of 10.

Rate of oviposition

Figure 1 shows the mean number of eggs laid per day by the 96 females. The daily rate of oviposition by individual females is shown in Figure 2. Though deviations occurred, as in Figure 2, the mean daily oviposition, as illustrated in Figure 1, follows a general pattern in all females. In most cases two or more peaks in daily oviposition can be discerned a few days apart, followed by a gradual

Table 1. Longevity and fecundity of females of *Atherigona soccata*

Reps.	No. of females	Fecundity			
		Range	Mean ± S.E.	Max. No. of eggs/ ♀ ♀/day	No. of eggs/ ♀ ♀/day
I	35	10–167	61.5±1.5	34	14.3
II	33	10–126	63.8±0.2	34	12.5
III	38	10–150	64.6±0.9	41	13.7
Total	96	10–167	62.8±0.9	41	13.5

Table 2. Effect of mating and adult food on the fecundity of *Atherigona soccata*

Adult food	Fecundity of mated females			Fecundity of virgin females		
	No. of ♀ ♀	Mean±SE	Range	No. of ♀ ♀	Mean±SE	Range
Brewer's yeast, glucose water	96	62.8±0.9	10–167	16	22.3±0.5	0–94
Glucose, water	16	25.6±0.4	0–50	19	3.4±.4	0–25

decline. The first eggs were laid a minimum of 2 days after the adult female emerged, the mean period being 4.7 days. Most of the males died earlier than the females, but no relationship was observed between the number of eggs laid per female and the amount of time spent with the male ($y=0.03x+13.47$; $r^2=0.03$). The maximum number of eggs was laid on the fourth day (Figure 1). In general, the females deposited most eggs early in their lives. Up to 41 eggs were laid in one day by one female during the first 10 days (Figure 2). The mean, however, was 13.8 eggs per female per day (Figure 3B).

The frequency distribution of the number of eggs laid per female per day is shown in Figure 3A. It was not exceptional, however, that after a few days of oviposition, the female completely stopped ovipositing and then resumed this activity later. Occasionally, large numbers of eggs were laid late in the female's life. This, as will be reported elsewhere, may be due to the withholding of eggs due to lack of suitable oviposition substrate. The data obtained from the last day of life of each female was excluded from the final analysis because it was not known exactly how long within that day the female was alive.

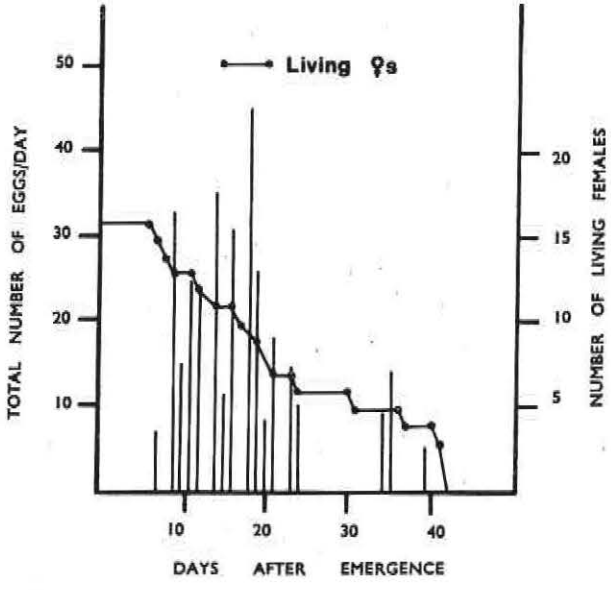

Figure 4. Longevity and rate of oviposition of 16 unmated females depositing in separate cages

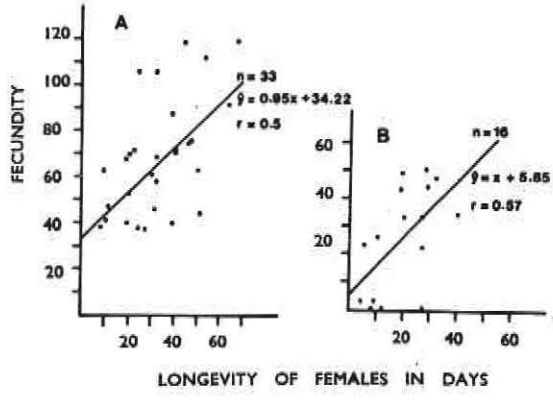

Figure 5. Correlation between longevity and fertility of
(A) 33 mated females
(B) 16 unmated females
The figures above the parenthesis give the number of eggs laid within a female's life span

Effect of mating on fecundity

Complete lack of mating had a significant effect on the fecundity of the females (Table 2), and the daily rate of oviposition (Figure 4). In the group of unmated females the oviposition was delayed for a minimum of 7 days after adult emergence, before the first eggs were laid. Most eggs were laid between 15 and 20 days after adult emergence. The highest peak in oviposition was reached 18 days after emergence (Figure 4). There was, however, no significant effect of lack of mating on the longevity of the females. The correlation between longevity and fecundity of the unmated females is shown in Figure 5.

Effect of adult food on fecundity

Table 2 also shows the influence of adult food on fecundity. The females maintained on glucose and water alone produced about half (25.6 ± 0.4) the number of eggs which were produced by females which had brewer's yeast in addition (62.8 ± 0.9). The effect was more significant in the virgin females, with the females fed on glucose and water alone producing only 15.3% (3.4 ± 0.4) of the number of eggs produced by those females which were fed on brewer's yeast (22.3 ± 0.5).

Adult longevity

The results of observations on longevity showed significant differences between males and females ($p<0.5$) reared under the same conditions. The 96 females maintained on brewer's yeast, glucose and water lived a mean of 32.6 ± 0.3 days with a minimum of 14 and a maximum of 73 days. The males maintained under the same conditions lived for only 25.6 ± 0.4 days with a minimum of 7 and maximum of 53 days (Table 4). The longevity of both males and females was affected mainly by adult food (Table 4).

In these observations, up to 167 eggs were recorded from one female, with a minimum of 62.8 eggs per female. Both the fecundity and the rate of oviposition were affected by adult food and mating. It is evident from these results, although not quantitatively proven, that the females require a proteinous food source before they can develop at least the first batch of eggs. Mating might also play a role in the release mechanism since the minimum pre-oviposition period was extended to 7 days in the unmated females. Another factor involved in this release mechanism might be the oviposition stimulus emanating from the host plant, since oviposition was reduced drastically by lack of a suitable host plant, in this case, the preferred host, sorghum.

Table 3. The influence of host plant on fecundity and longevity of *Atherigona soccata*

	No. of ♀♀	Sorghum alone	No. of ♀♀	Sorghum and other grass species
Fecundity	38	64.6 ± 0.9	47	27.0 ± 0.6
Longevity in days (Mean±SE)	38	35.8 ± 0.4	47	36.4 ± 0.3

Table 4. Adult longevity of *Atherigona soccata*

	Female longevity in days			Male longevity in days		
	♀♀ No.	Range	Mean±SE	♂♂ No.	Range	Mean±SE
Brewer's yeast, glucose, water	96	14–73	32.6 ± 0.3	31	7–53	25.4 ± 0.4
Glucose, water	16	4–40	19.8 ± 0.2	13	4–26	13.8 ± 0.6

Effect of host plant on fecundity

As would be expected, fecundity was also greatly influenced by the host plant. An unsuitable host plant reduced the female's fecundity but did not affect longevity (Table 3). The females enclosed for intervals of 5 days on sorghum and separately on four other grass species, *D. scallarum*, *R. exaltata*, *S. verticillata* and *P. maximum*, had a mean fecundity of about half that of the females which were enclosed on sorghum throughout (Table 3).

The fecundity and longevity of *A. soccata* vary considerably from region to region. At Udaipur in India the fecundity varied between 20 and 25 eggs in a life span of 12 to 14 days. The daily rate of oviposition was 1 to 2 eggs. In Thailand, the adults are known to live an average life span of 13.6 days when reared on sorghum. These figures are extremely low in comparison to the 62.8 eggs per female in a life span of 32.6 days observed during the period of our study. The mean daily rate of

oviposition per female was 13.5 eggs, with a maximum of 41 eggs. With this oviposition potential, shootfly can be a very serious pest during periods with adequate rainfall.

Ovipositional behaviour and host plant preference of the sorghum shootfly, *Atherigona soccata* Rond. (Diptera, Anthomyiidae)

K. Ogwaro

The distribution of eggs on sorghum by shootfly and the host preference for oviposition of *Atherigona soccata* Rond. were studied. The female distributed her eggs more or less evenly on sorghum leaves, laying one or two eggs per leaf. Under field conditions eggs were most frequently laid on the third leaf, followed by the second leaf, while under insectary conditions the second leaf was preferred to the third. *Sorghum bicolor* was markedly preferred to other graminaceous plant species, viz. *Digitaria scallarum*, *Rottboellia exaltata*, *Setaria verticillata* and *Panicum maximum*.

The ovipositional response of the sorghum shootfly, *A. soccata* on some promising resistant lines of sorghum has been the subject of investigation by Jotwani and his co-workers in India. Jotwani and Srivastava observed that although some lines may be considered as possessing a high degree of resistance against the sorghum shootfly, none of the lines tested was immune or completely resistant against the shootfly. The factors causing the non-preference for oviposition have not been identified yet, although there are speculations that certain chemicals and leaf structures may be involved. To understand the mechanism of recognition of the various host plant types by the ovipositing female, the study of sensory physiology is required. The present work is, however, an additional attempt to clarify some aspects of ovipositional behaviour on an experimental basis.

Ovipositional behaviour was studied both under free air greenhouse conditions and in the field. For the greenhouse observations, young plants from 3 to 7 leaf stages were enclosed in transparent plastic oviposition cages measuring 23 × 11 × 11cm containing single pairs of male and female flies. The plants of differing ages were included for the purpose of studying the leaf stage preference for oviposition. The flies were maintained on brewer's yeast, glucose and water. The plants were examined daily at 17.00 hours for eggs. The cages where eggs were deposited were replaced by others containing healthy plants. The plants were changed after every 4 days. The eggs on the infested plants were counted and their distribution recorded. Longevity and fecundity of the adults were determined when they died. The variety of sorghum used was Serena, which is a variety developed simultaneously in Uganda and Tanzania by Dogget, as a commercial variety for East African lowland areas (1,530 metres). It shows appreciable levels of resistance to *A. soccata*, witchweed (Striga) and leaf diseases.

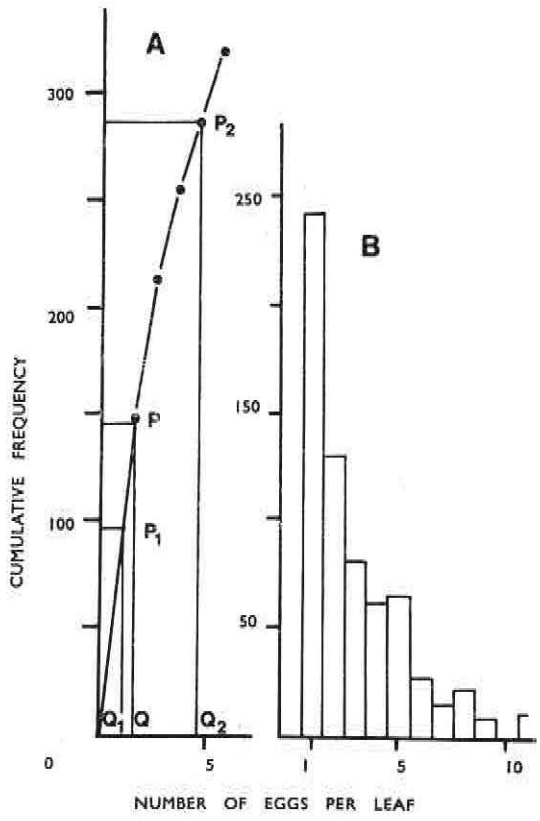

Figure 1. Frequency distribution of number of eggs per leaf (B), the cumulative frequency (A) shows distribution range of between 1 and 4.5 eggs per leaf and a mode of 1.5 eggs per leaf

Different numbers of plants per cage were used to determine the effect of plant density on egg distribution. To study the relative ovipositional preference for different host plants, sorghum (Serena) and four other graminaceous grass species, viz: *D. scallarum*, *R. exaltata*, *S. verticillata* and *P. maximum* were introduced into the cages in isolation of each other and changed every 5 days (in sequence of the list) to provide alternative situations. In another set of experiments on ovipositional preferences, sorghum and each of the four other species were enclosed in the same cage to provide a choice situation. The four wild grass species were chosen for this study because they were often found to be infested by *A. soccata* in the field. Several individuals of *A. soccata* were reared from *Setaria* and *Digitaria* and a few materials from *Panicum* and *Rottboellia*.

The field study was conducted by removing all the infested plants from particular field plots every other day. The eggs on each plant were counted and their distribution recorded.

Adult behaviour at oviposition site

Before ovipositing, the female moves from plant to plant and leaf to leaf, probing the leaf surface with her fore and hind legs as well as with the ovipositor. When

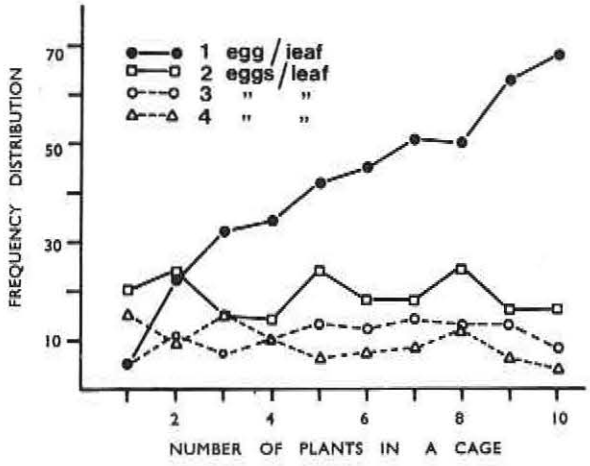

Figure 2. The effect of plant density on the distribution of eggs

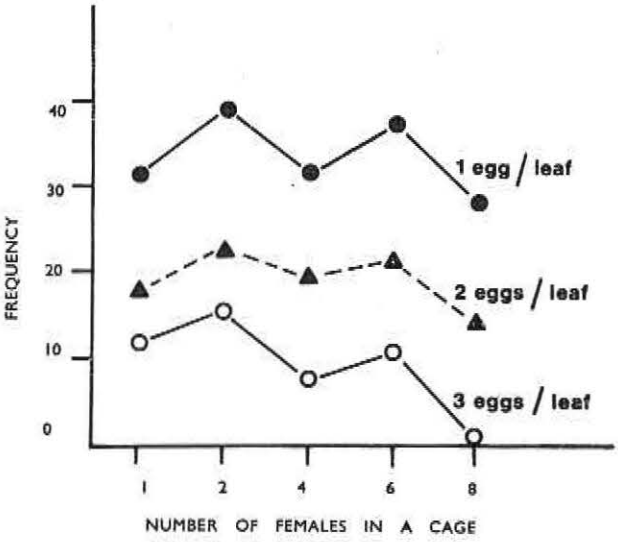

Figure 3. The effect of increased numbers of females in a cage on the distribution of eggs

finally the right plant and oviposition site is selected, she presses the ovipositor against the leaf surface and lays her eggs, usually parallel to the mid-rib. The female moves upwards vibrating the ovipositor while at the same time pressing it against the surface. This ensures that the eggs are cemented firmly on to the surface.

There often seems to be an attraction to a particular area in that, after the female has laid the first egg, she may move around and return to lay the next egg near the first one. This behaviour, as is shown below, is due to the shortage of oviposition substrate in the cages and does not happen under field conditions where the female has several plants and leaves to select from. In the greenhouse, many eggs may be laid in a line and sometimes close and parallel to each other on the same leaf.

Distribution of eggs on sorghum

There were one (10.2%) or two (1.6%) and rarely three (0.2%) eggs per leaf under field conditions. Most of the leaves in the samples did not have any eggs (88%) (Table 1). Under greenhouse conditions, however, up to 29 eggs were recorded on one leaf on a four leaf stage plant. The frequency distribution of numbers of eggs per leaf is shown in Figure 1B. This unusual number was due to the limited quantity of oviposition substrates offered (2–3 plants per cage), but the mean number of eggs per leaf was still clearly one, as shown in the cumulative frequency of Figure 1A.

There is a positive correlation between the distribution of one egg per leaf and the number of plants in a cage ($y=4.33x+6.6$; $r^2=0.89$), but a poor correlation between two eggs per leaf ($y=1.3x+7.9$; $r^2=0.36$), while there was no correlation at all between three ($y=0.18x+5.8$; $r^2=0.02$) or more eggs per leaf and the number of plants in a cage (Figure 2). From these experiments it appears that the distribution of more than one egg per leaf is unlikely in a situation where there is freedom of choice of host plants for the ovipositing female. It is not surprising, therefore, that under field conditions there were only one or two eggs per leaf.

Increasing the number of the ovipositing females in a cage while keeping the number of plants constant still showed that most of the leaves had one egg each (Figure 3). This means that some mechanism stimulating an even distribution must be operating. The female recognizes that there is already an egg.

Choice of oviposition sites

The behaviour pattern which leads to the final selection of oviposition site has been described above. The choice of oviposition sites is different under field and insectary conditions. In the insectary, the second leaf order was most preferred for oviposition, followed by the third, first and fourth, with 52.5, 28.6, 16.8 and 2.1% of total eggs deposited respectively.

Under field conditions the third leaf order was most preferred, followed by the second, fourth, fifth, sixth, first and then seventh, with 54.1, 28.5, 13.3, 3.2, 0.5, 0.4 and 0.1% of total eggs deposited respectively. A graphical representation of the ovipositional preference for the different leaf orders is shown in Figure 4. Besides the different leaf orders, the female has a choice of either the upper or lower leaf surface. The lower leaf surface was preferred to the upper one, with only 9.8% of the total number of eggs being laid on the upper surface. Considering the total mean number of eggs per leaf, it can be seen that twice as many eggs per leaf were laid on the lower surface (3.8) as on the upper surface (1.9). Most of the eggs deposited on the upper surface were on the youngest leaf (Table 2).

Host plant preference

When only one type of shoot is presented to the gravid female, the absolute number of eggs laid, the proportion of the shoots infested with eggs and the frequency of the number of observations during which eggs were laid

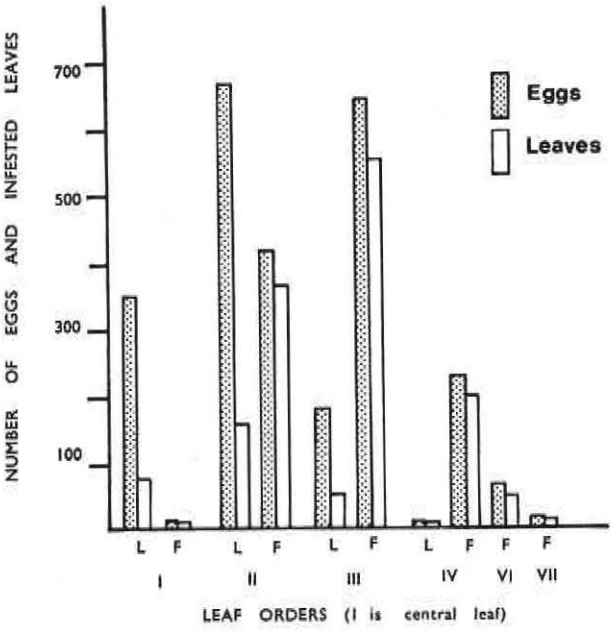

Figure 4. Leaf order preference for oviposition under green house (L) and field (F) conditions

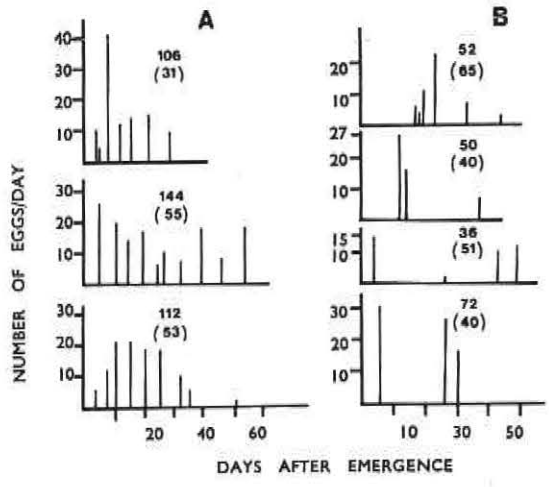

Figure 5. Daily distribution of eggs by individual females; A, with sorghum alone present throughout and B, with sorghum alternating with four other grass species at 5 day intervals. The figures above parenthesis are number of eggs laid in a female's life span

could indicate preference levels. This was based on the consideration that the female will not deposit any eggs in the absence of a suitable oviposition substrate. Table 3 summarizes the results of these experiments. The females deposited frequently on sorghum, but *D. scallarum*, *R. exaltata* and *S. verticillata* were found to be only marginal oviposition substrates. Eggs were laid on these grass species at very irregular intervals. There were 3, 1 and 2 positive responses out of 38, 18 and 37 observations on *D. scallarum*, *R. exaltata* and *S. verticillata* respectively. No eggs at all were laid on *P. maximum* under these conditions.

There was restraint in oviposition when species of grass other than sorghum were presented to the ovipositing females. They stopped ovipositing until sorghum was re-introduced. As many eggs as during the previous exposure were sometime deposited during this second exposure period, indicating the ability of the gravid female to withhold her eggs in the absence of suitable hosts, and deposit them when a suitable host is again available (Figure 5). The host plant selection experiment conducted with 23 females ovipositing in separate cages, with the five hosts changed every 5 days, showed that a decline in oviposition, which normally started a few days after adult emergence, did

Table 1. Egg distribution on the different leaf orders under field conditions

No. of eggs per leaf	Leaf Orders							Total	%
	I	II	III	IV	V	VI	VII		
0	1534	1054	456	1340	1498	1537	1537	8956	88.0
1	6	324	483	174	42	8	1	1038	10.2
2	1	49	81	24	5	1	0	161	1.6
3	0	3	9	4	4	0	0	20	0.2
Total	1541	1430	1029	1542	1549	1546	1538	10175	100.0

Table 2. Leaf surface preference for oviposition by *Atherigona soccata* under greenhouse conditions

Leaf order	Upper leaf surface			Lower leaf surface		
	Total No. of eggs	Total No. of infested leaves	No. of eggs per leaf	Total No. of eggs	Total No. of infested leaves	No. of eggs per leaf
I	144	55	2.6	367	96	3.8
II	115	81	1.4	1481	378	3.9
III	33	17	1.9	838	224	3.7
IV	5	4	1.3	59	17	3.5
Total	297	157	1.9	2745	715	3.8

not start until 23 days after emergence of the females (Figure 6). The number of eggs laid per female per day when sorghum was introduced after the 5 day interval was not significantly different from the number deposited under normal situations with sorghum being available throughout (Figure 7). The mean number of eggs laid per female per day on the introduction of sorghum in this experiment was 12.9, which is not significantly different from the number laid per female per day (13.8) when sorghum was available throughout. The results of the choice experiments are shown in Table 4. Again there is a definite choice of *S. bicolor*. The next preferred was *D. scallarum* and then *S. verticillata*. No eggs were laid on *R. exaltata* and *P. maximum* (Table 4).

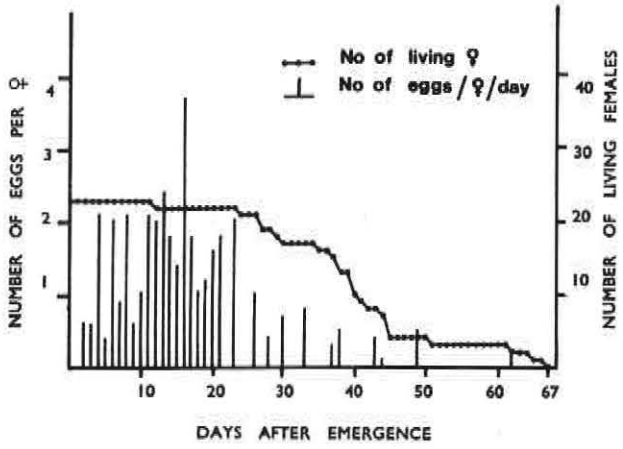

Figure 6. Longevity and rate of oviposition of 23 females depositing in separate cages in a situation where sorghum was introduced alternatively with 5 other grass species at 5 day intervals

Table 3. Relative ovipositional preference of *Atherigona soccata* expressed as percentage of the total number of eggs laid on the different host species, percentage of the number of infested plants and the percentage of the number of favourable responses (non-choice experiment)

Host Plant species	No. of observations	% of infested plants	% of positive responses*	% of no. of eggs
D.scallarum	38	5.3	7.9	3.2
P.maximum	29	—	—	—
R.exaltata	18	1.4	5.6	0.2
S.verticillata	37	1.4	5.4	3.7
S.bicolor	43	78.5	76.2	92.9
Total	165	88.5(146)	23.0(38)	100.0(871)

*The % of exposures during which eggs were laid
( ) Figures in parenthesis are the totals of infested plants, positive responses and number of eggs respectively.

Table 4. Relative ovipositional preference of *Atherigona soccata* for sorghum and other grass species (two-choice experiments)

	The number of eggs laid on:			
Female Number	*S.bicolor*	*S.verticillata*	*D.scallarum*	Total
1	85	2	2	89
2	83	6	11	100
3	1	0	0	1
4	86	0	5	91
5	0	0	0	0
6	129	0	2	131
7	73	1	4	78
8	56	0	4	60
9	79	0	0	79
10	34	0	0	34
Total	626	9	26	663
%	94.4	1.4	4.2	100.9

S.bicolor/S. verticillate; $t=4.02$, $P<0.1$; *D.scallarum/S.verticillata*
S.bicolor/D. scallarum; $t=3.87$, $P<0.5$; $t=6.48$, $P<1.0$

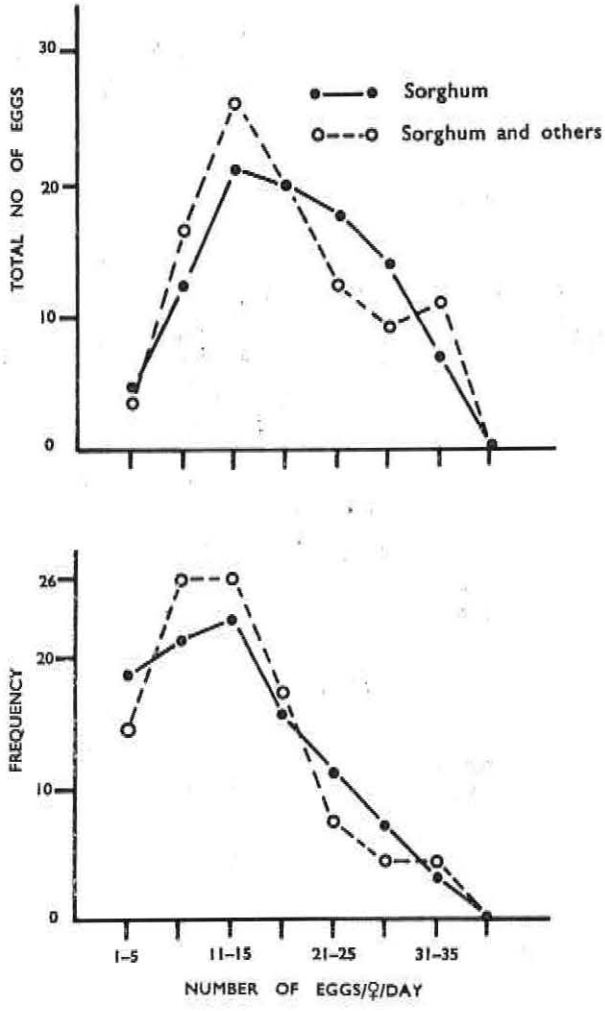

Figure 7. Percentage total number of eggs (above) and percentage frequency of number of eggs laid per female per day (below)

Discussion

In these experiments, no eggs were laid when flies were denied access to host plant material, and very few eggs were deposited when the flies were presented with marginally suitable host plants. These results show that although some flies may deposit small numbers of eggs indiscriminately under excessive oviposition strain, under normal conditions oviposition occurs only on perception of appropriate stimuli from host plant material. To minimize the chances of indiscriminate laying of eggs, the various host plants tested were changed every 5 days. By so doing the flies were subjected to minimal oviposition strain.

The definite preference for sorghum above other grass species indicates the presence of an ovipositional attractant within the sorghum plant. This could be physical or chemical. In another Anthomyiid fly, *Hylemya brassicae*, Nair and McEwen have demonstrated that mustard oil, allyl isothiocyanate (AITC), stimulates the flies to greater activity and at the same time serves as an attractant. Sinigrin and four other glucosinolates (mustard oil glucosides) tested by these authors induced oviposition, but the AITC did not induce oviposition in the absence of glucosinolates. The common nutrients such as glucose, casein, wheat germ oil, and B-vitamin mixtures did not influence oviposition. It was suggested by Nair and McEwen that oviposition preference in *H. brassicae* is governed by the presence of some "key" glucosinolates and the absence of inhibitory chemicals.

In the frit fly, *Oscinella frit*, the oviposition sites are known to change as the plants develop. Ibbotson earlier showed that the growing tissues of young plants produce chemical substances that stimulate egg laying in the frit fly, *O. frit*, and that the tactile receptors on the ovipositor are also involved in the perception of such stimuli. In the present experiments it was observed that the female surveyed the leaf surface with her fore and hind legs as well as with the ovipositor before she finally selected a spot to deposit her eggs. Most of the eggs were deposited on the lower surface of the second and third leaf orders below the central shoot.

Ponnaiya, studying *A. soccata* in India, also found the lower leaf surface most preferred with only 0.2% deposited on the upper side. He observed that the female skipped off the seedling which was already attached and laid its eggs on the uninfested leaves resulting in the distribution of one egg per leaf. Ponnaiya suggested that where more than one egg per leaf were laid these might be by more than one female. This seems to imply that the female can recognize its own eggs and disregard the presence of eggs laid by other females. This suggestion has been confirmed by our observation where increased number of females in a cage showed a non-random distribution of eggs.

Investigation of the factors affecting oviposition on sorghum seedlings of various cultivars as well as in alternative host plants is therefore of great importance in the understanding of resistance to shootfly. It will also be of great interest to investigate whether there is any similarity in the properties of sorghum and in the response of *A. soccata* in comparison to the situation described for *O. frit* and *H. brassicae*.

MOSQUITO RESEARCH

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The regulation of preimaginal populations of *Aedes aegypti* L. "type form" on the Kenya Coast

R. Subra

On the Kenya Coast *Ae. aegypti* "type form" breeds in domestic containers (jars and drums) where people store water for domestic purposes. In 1976 it had been observed that it was possible to divide these containers into three types: high breeding, low breeding and intermediate type. The hypothesis was put forward that food was the main regulatory factor of preimaginal populations. Several experiments were designed to demonstrate this.

(1) By adding food into containers where this species is used to breed.
(2) By adding larvae, but no food into these containers.

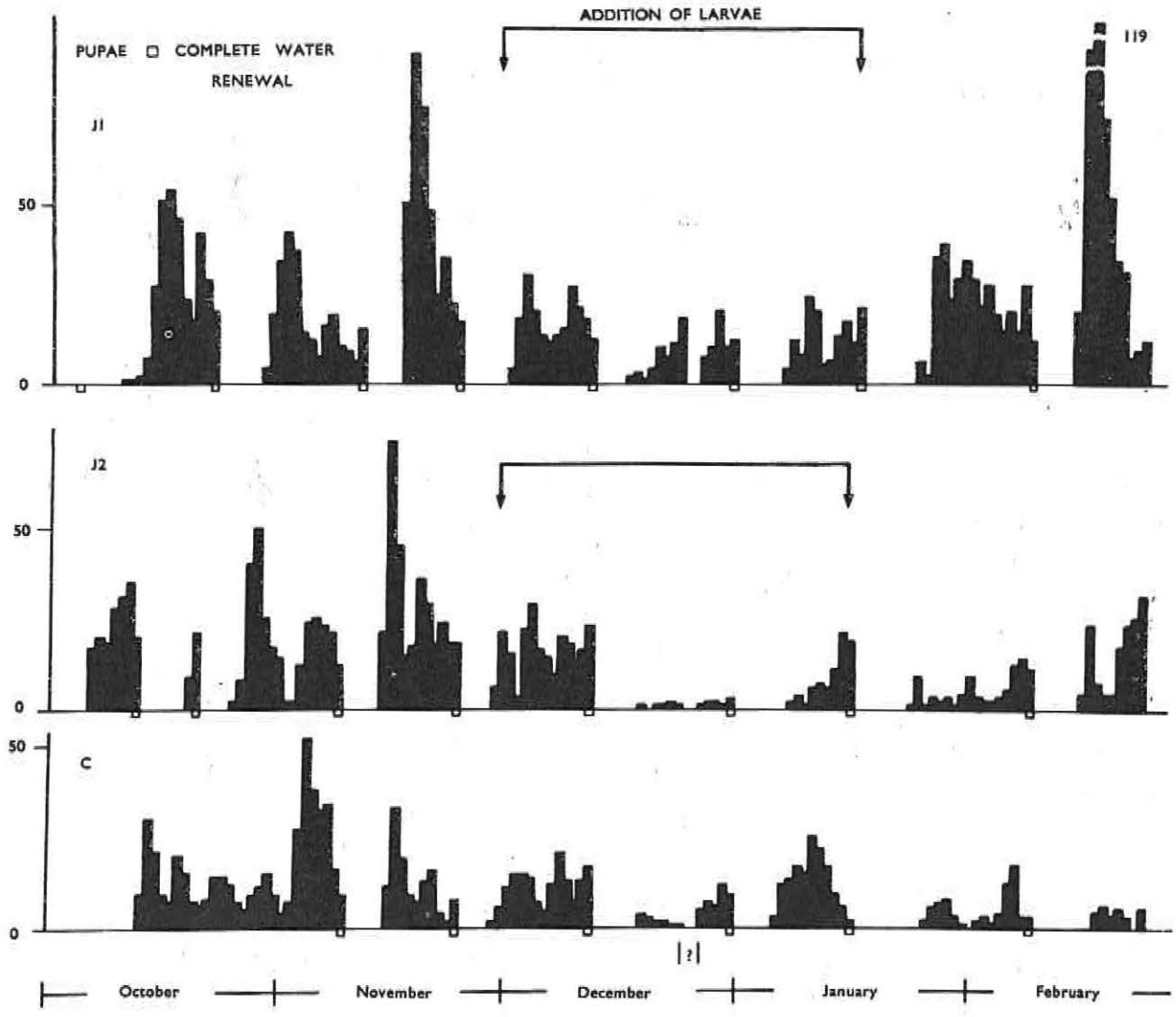

Figure 1. Influence of larval addition on pupal densities of *Aedes aegypti* in high breeding containers

(3) By studying oviposition behaviour in order to check if there was any difference between different types of containers (low and high breeding containers).

The first experiment was completed in 1976. The two other experiments were a major part of research activities performed in 1977.

Influence of larval addition on pupal densities variations in different types of containers

The present experiment was designed by selecting four jars, two belonging to the high breeding type (numbered 1 and 2) the other two to the low breeding type (numbered 3 and 4). In addition, several other jars (numbered C, CI, C2 and C3) were kept for control. This experiment ran for about 4½ months, in 3 phases: preliminary observations, addition of larvae and follow-up.

During the second phase 400 first instar larvae were introduced daily into the four selected jars. All observations were made daily.

In the two high-breeding jars the introduction of larvae did not promote any increase in pupal densities (Figure 1).

The results obtained with the two low-breeding jars are more heterogeneous (Figure 2). Jar No: 3 did not show, during larval addition, any significant increases. In jar No. 4 the addition of larvae has been followed by prominent increase in pupal densities. One could prematurely conclude that in this jar there was a larval deficit. But when looking at the graph of this jar for the whole experiment it appears that there was no other sharp increase in the pupal numbers, even during larval addition. Several weeks after the end of larval addition there was a sudden increase in pupal numbers, less

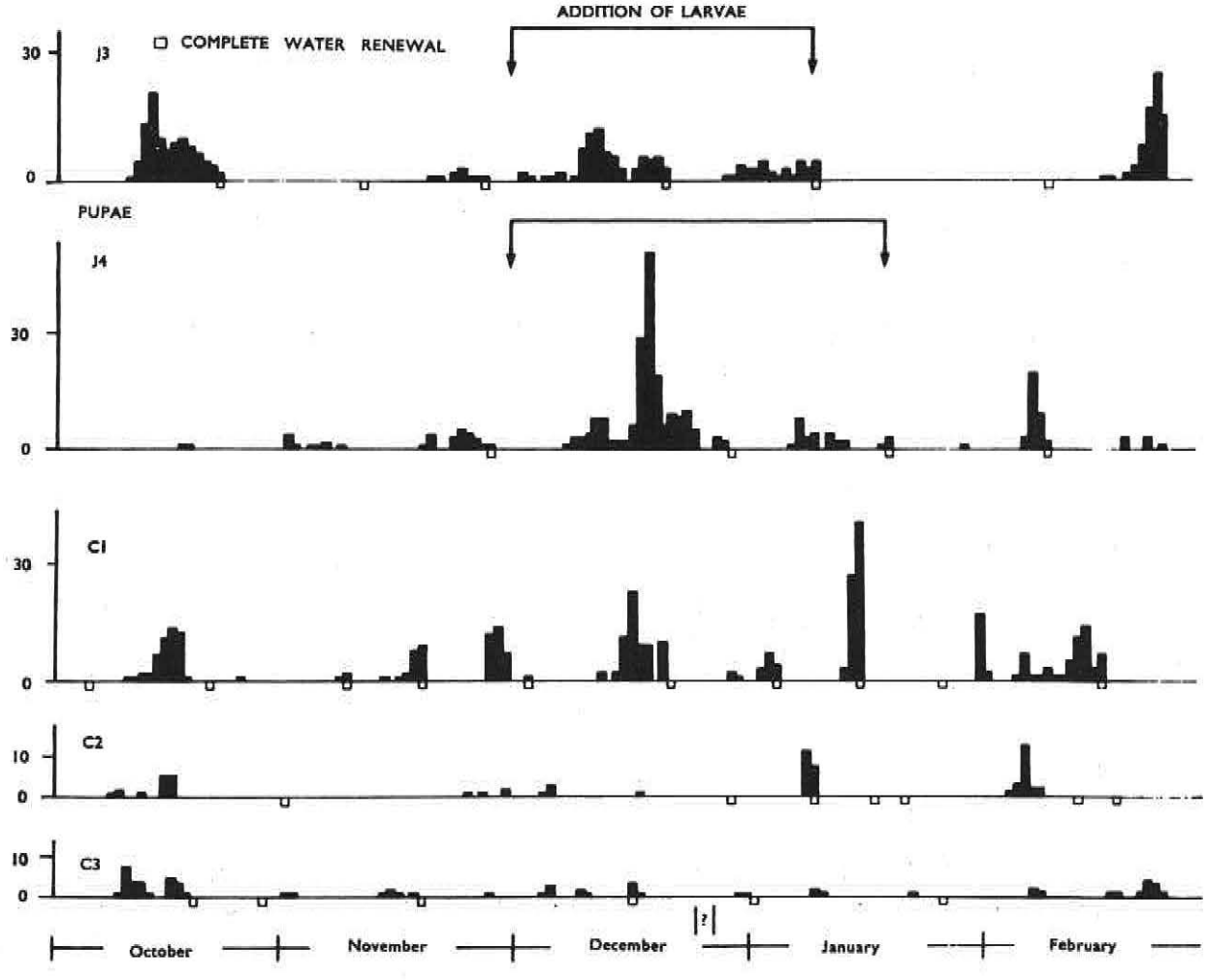

Figure 2. Influence of larval addition on pupal densities of *Aedes aegypti* in low breeding containers

than the former one, but nevertheless prominent. When looking at the control jars, it appears that while some of them are very stable over several month periods, in other jars there are sometimes sudden bursts preceeded and followed by long periods of low density. Such increases in these jars, as well as in jar No. 4, could be explained by an accidental introduction of food into these jars.

In conclusion, the addition of larvae does not usually have any effect on the densities.

Oviposition

The aim of this experiment was to prove if there were any differences in the attractiveness of the different jars for gravid females, that is, to check if jars with intense breeding were more attractive than other jars.

A positive answer would have proved that differences in pupal densities may be explained, not only by the availability of food but also by the amount of eggs laid in the different containers.

Gravid females were given a choice between five tins, two of them containing water from two highly populated jars, two others containing water from two jars with low-breeding populations, and the last one containing tap water as a control. An ovistrip was fixed inside each of these tins for collecting the eggs. Tins were set in square metallic drums. We had four of these metallic drums set in four different houses in the village. Water samples were renewed every day and observations were made daily.

Our observations ran for 80 days. We had enough data to assume that over a long period of time there is no egg deficit in the low-breeding jars. Thus oviposition does not play a major role in the regulation of preimaginal densities.

Overcrowding under some conditions may be an important regulatory factor. For *Ae. aegypti*, in the village where we made our observations, breeding even in highly populated jars never reaches a crowded situation, and daily water renewal, even if not complete, eliminates a part of potential overcrowding factors. According to our observations therefore, and considering certain other factors, we think that food is the main regulatory factor of the preimaginal stages of *Ae. aegypti* "type form" breeding in the rural areas of the Kenya Coast.

TERMITE RESEARCH

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Observations on flight patterns and other characteristics of *Macrotermes* nests

J. P. E. C. Darlington

Two types of *Macrotermes* mounds occur in Kajiado district; the closed type of mound found arround Kajiado township, and the open type found in Amboseli and on the floor of the Rift Valley. These were briefly described in the 1975 ICIPE Annual Report and were at that time tentatively attributed to differences in soil type affecting the building behaviour of the same species of termite. Information obtained since then makes it very unlikely that this is the true explanation.

We now know that the open type of nest occurs at lower altitudes than the closed type, but that there is a zone of overlap in which both types of mound occur together on the same soil type. Contrary to earlier ideas, there seem to be no true intermediates in internal structure. This makes it more probable that the two nest types represent two different species or subspecies.

To try to clarify this matter, close observation was kept (in collaboration with M. Lepage) through the short rains at Bissel, a small town about 30km south of Kajiado, which is in the overlap zone where both nest types occur. Observations of flight holes in previous years had shown that both types of nests produced alates during the short rains. What we wished to test was whether or not the actual alate flights were synchronous so that interbreeding could occur.

The first heavy rain occurred on 29 October 1977 but very few flight holes were built at that time. A large open mound (no. 362) was fumigated and dug on 16 November, and 14,784 alates were recovered. On 17, 18, 19 November a total of 19.2mm of rain fell, and flight holes began to appear on both open and closed mounds. On 21 November some activity was observed on open mounds at dusk, with flight holes being opened and workers emerging onto the mound surface. No such activity was observed on closed mounds. Two open mounds were kept under continuous observation, and they remained active all night, with many thousands of workers streaming over the external surface of the mound. At about 3am alates began to emerge, and a very heavy flight continued until after 5am. A survey of nests the next day showed that almost all the open mounds had fresh flight holes and discarded wings nearby, whereas of the closed mounds, few had flight holes and none had discarded wings.

A heavy shower on 24 November obliterated all existing flight holes. None were rebuilt on open mounds, but on closed mounds even more were built than before. An open nest (no. 366) which had had many flight holes before the flight was fumigated on 2 December and not one alate was found inside. A closed mound (no. 365) with flight holes fumigated at the same time contained a large brood of alates.

On 19–20 December steady rain fell to a total of 25mm. At dusk on 20 December alates flew heavily from closed mounds over a period of half an hour, after which worker activity rapidly subsided. Smaller

Table 1. Mean fresh and dry weights of *Macrotermes* alates. Mean weights in mg (number of individuals weighed)

	Fresh Weights				Dry Weights			
	Males		Females		Males		Females	
Kajiado flight 7 December 1975 (*1)	171	(200)	181	(200)				
Kajiado flight 26/30 November 1976	168	(82)	178	(82)	91	(85)	96	(107)
Open mound 20 km S of Kajiado 11 January 1977 (*2)	197	(7)	223	(13)				
Kajiado flight 21 December 1977					103	(100)	107	(100)
Open mound at Bissel 17 November 1977					138	(110)	148	(110)
Closed mound at Bissel 20 December 1977					130	(110)	132	(118)

*1 Weighed by G. Bühlmann
*2 Collected by O. Bruinsma and P. v.d. Werff

flights are believed to have occurred both before and after this date.

There was thus a gap of one month between the main flights of the open and closed mounds, with the former responding more rapidly to the particular pattern of rainfall experienced this year. Even if the dates had coincided, the times at which the alates flew were separated by eight hours (7pm to 3am). Thus there seems to be an effective reproductive isolation mechanism in this area of overlap, which strongly supports the idea that the two mound types belong to different taxa. The pattern of piecemeal flights at a restricted time of day over an extended period observed in the closed mounds at Bissel is much the same as that recorded for the past three seasons in the closed mounds at Kajiado. The pattern for the open mounds of a single, very heavy, extended flight appears to be quite different.

One fallacy has come to light as a result of this work. From the meagre data collected in 1976 it had been thought that the alates from open nests were heavier than those from closed nests (Table 1). In the 1977 flight season alates from both open and closed nests at Bissel were found to be equally heavy, while those from Kajiado were lighter (Table 1). Thus it appears that the difference is between geographical areas, not nest types.

In January 1977 a series of nests was excavated by O. Bruinsma and P. v.d. Werff in the zone of overlap between the two mound types. Pairs of nests were selected, one open and one closed, of roughly the same size, on the same soil and in the same habitat. The results will be reported in detail elsewhere. The opportunity was taken to make a long series of measurements of the dry weights of adult sterile castes. There were variations between individual nests, which on the basis of other observations are tentatively attributed to differences in the total mound population. Workers and minor soldiers showed only slight differences between open and closed nests, but in major soldiers the mean weights were clearly different (Table 2). This in itself does not prove that genetic differences exist, because the differences in nest construction may affect the internal temperatures and thus the development of the brood. Work on heat relations in the two nest types is planned for 1978.

The taxonomy of our *Macrotermes* species is thus somewhat confused. It seems probable that the open type of mound belongs to *Macrotermes subhyalinus* proper, while the closed mound is a related species which we refer to at present as *Macrotermes* near *subhyalinus*. The discrete geographical distribution and very narrow zone of overlap suggest that the two species occupy almost the same ecological niche. The closed mound species closely resembles *Macrotermes michaelseni* (Sjöstedt), but is far outside the previously known range of that species. (The nearest recorded occurrence is in Mozambique.)

Table 2. Comparison of weights of major soldiers from nine "matched" pairs of open and closed mounds. Mean dry weight in mg (number of individuals weighed)

Closed Mounds		Open Mounds	
21.4	(113)	25.4	(110)
20.3	(24)	31.5	(106)
16.2	(111)	31.3	(25)
23.6	(17)	35.2	(110)
22.2	(110)	33.4	(110)
25.4	(74)	35.1	(110)
21.0	(6)	35.7	(110)
24.1	(110)	27.9	(77)
16.9	(110)	25.3	(110)

Foraging and feeding of *Macrotermes*

M. G. Lepage

The aim of this study is to assess the food intake and grass preferences of *Macrotermes* near *subhyalinus* in semi-arid rangelands (Kajiado—Kenya), in order to provide a better management of these pastures. The experiments are carried out within or nearby a 1ha exclosure, 8km south of Kajiado, where there is a small weather station. Grass biomass and litter production are checked monthly and the large mammals (livestock and game) seen in the vicinity are recorded daily.

Rain falls in two seasons (March-April-May and November-December), but, as shown in Figure 1, a wide variation occurred between 1976 (306mm) and 1977 (650mm).

The standing-crop followed this rainfall pattern (cf. Figure 1) and decreased almost continuously since November 1975 (2550kg/ha) to 600–800kg in November 1976. Following the high rainfall in April-May 1977, the standing-crop peak reached 5430kg in June 1977. The litter (Figure 1) also decreased from 1150kg/ha in January 1976 to 100–200kg in November 1976.

As stated previously (ICIPE Annual Report, 1976), *Macrotermes* shows a daily and seasonal pattern. Figure 2 records the seasonal variation of foraging activity throughout 1976 and 1977, expressed as monthly averages of holes utilized per m2 and per 24 h-cycle. A general pattern of the activity can be described: one peak in February-March, related to temperature increase and to food-availability decrease, and one peak in June-August, related to the maturation of the sexual castes.

In order to quantify the total grass intake of *Macrotermes*, several methods are used, the provisional results fluctuating within a reasonable range: 730 to 1628kg per ha and per year. In comparing the food intake in April-November 1976 and 1977, it seems that the monthly quantities were 25–30% lower in 1976 (71–101 kg/ha as compared with 104–136). These differences probably show the food supply difficulties of *Macrotermes* towards the end of 1976 (by this time, both standing-crop and litter were near their minimum). The analysis of food taken between standing-crop (G) and litter (L) backs up this hypothesis: in 1976, the standing-crop constituted an increasing proportion of food intake (in November 1976, L=26.1%, G=73.9%, while in November 1977, L=65.6%, G=34.4%). This statement shows how *Macrotermes* could become a pest: if the food availability decreases below a certain minimum level, the termite switches its consumption from the litter to the standing-crop. Thereafter, the final step is root consumption.

Since one of the objectives of the study is to establish the relative importance of termites and mammals in the ecosystem, the first results already show the main characteristics of this comparison.

Mammal abundance is shown in Figure 2 (daily biomass, monthly averages). This abundance correlates positively with the rainfall. Over 20 months, the mean daily biomass for cattle, sheep and goats is 37 tons (=82 Animal Units), and 14 tons for game animals (=31 A.U.). Experiments are in progress to assess a density per ha from these figures. The relative proportions of domestic and wild animals are 88–12 in the rainy season and 67–33 in the dry season.

The total grass intake of mammals and *Macrotermes* is of the same magnitude: at the peak of the standing-crop, the mammals took from 587kg/ha in April 1976 to 945kg in June 1977. A detailed comparison is outlined in Table 1. In general, the proportion taken by mammals decreases when this proportion increases regularly (3–8% per month) for *Macrotermes*. The important role of the termite is shown in Table 1: at a critical time for the ecosystem (November 1976), *Macrotermes* took more than 30% of the total grass biomass.

However, the food quality differs in both cases. Mammals feed mainly on the standing-crop and on green grass

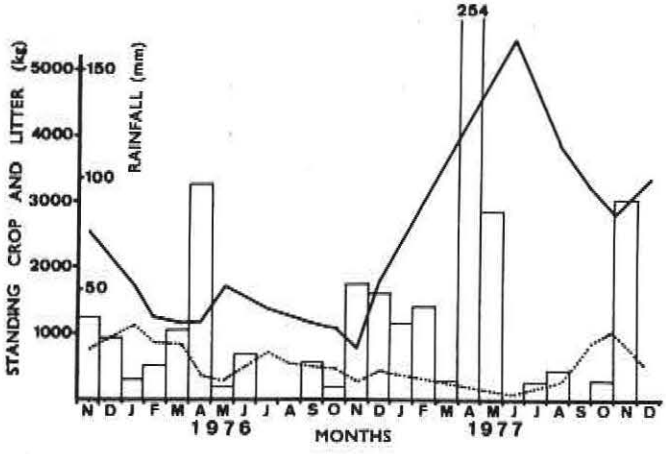

Figure 1. Standing-crop (continuous line) and litter (dotted line) in kg/ha related to the monthly rainfall (histogram)

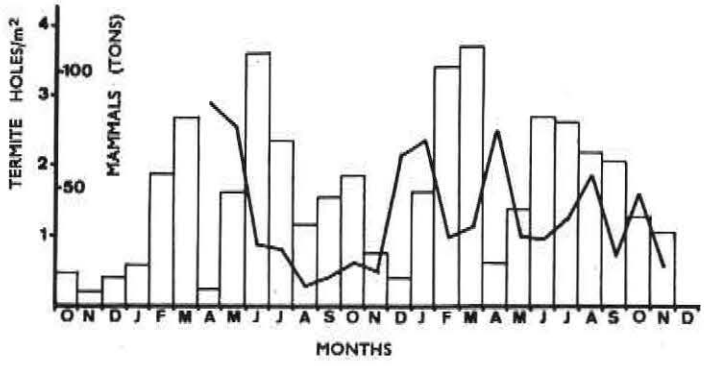

Figure 2. *Macrotermes* foraging activity (holes open per m²-histogram) and mammal abundance (biomass in tons—continuous line)

Table 1. Food intake of mammals and *Macrotermes* near *subhyalinus* (percentages of the total standing-crop (G) and litter (L) available)

Month	Mammals			*Macrotermes*			Total of both		
	G	L	G+L	G	L	G+L	G	L	G+L
VIII—76	—25.9	+ 3.1	—15.9	— 7.6	—28.3	—14.7	—33.5	—25.2	—30.6
IX—76	—23.2	+ 5.4	—15.6	—14.0	—40.1	—21.0	—37.2	—34.7	—36.6
X—76	—20.8	—13.3	—18.9	—19.3	—23.0	—20.3	—40.1	—36.3	—39.2
XI—76	—12.1	—33.3	—16.7	—28.6	—37.0	—30.4	—40.7	—70.3	—47.1
VIII—77	—23.9	+21.6	—21.1	— 7.9	—40.6	—10.2	—31.8	—19.0	—31.3
X—77	—17.0	—10.4	—16.9	— 7.8	—65.0	—21.7	—24.8	—75.4	—38.6
XI—77	—18.9	— 5.0	—16.3	—11.0	—60.3	—25.4	—29.9	—65.3	—41.7

when available, (in May 1976, the grass proportions green-dry were 45.8–54.2 inside plots protected from mammals, and 23.9–76.1 outside). The decrease of litter when mammals are present is not yet explained. On the contrary, *Macrotermes* prefer the litter on the ground. But, as shown above, if this litter becomes scarce (in a period of long drought or during the rainy seasons, Figure 1), the termite consumes a noticeable proportion of the standing-crop. It is too early to know if the consumption of mammals and termites have a marked effect upon grass composition. Termites food preferences particularly have not yet been studied in detail. From early experiments, species diversity increases from termite-protected plots to plots exposed to all herbivores. Mammal consumption seems to result in an increase of the species *Cynodon dactylon*, *Pennisetum stramineum* and *Microchloa kunthii* and a decrease of *Themeda triandra*.

Macrotermes—caste differentiation

G. Bühlmann

Descriptive morphometric studies have demonstrated that the current theories about caste formation in the related species *Macrotermes bellicosus* can also be applied to the *Macrotermes* near *subhyalinus* species which is under investigation in this laboratory. For studying the dynamic aspects of mechanisms and factors involved in caste differentiation however, it is necessary to do experimental investigations on living termites under laboratory conditions.

Our efforts to rear *Macrotermes* eggs, larvae or nymphs in the laboratory were of little success as long as we used groups of termites which were collected in the field to bring them up. However, much better results were obtained by using young imagoes for rearing eggs and larvae. It does not matter whether they have to care for their own eggs or larvae, or if they receive them from another colony. It is also not important to which sex the foster parents belong. Pairs of males are as suitable for fostering as are pairs of virgin females. In both cases no fertile eggs are produced, which is an advantage over male-female pairs, where it is virtually impossible to distinguish between their own and introduced eggs. Fostering by single animals is also possible but less suitable for our purpose, since mortality is greatly increased when there is no partner for mutual grooming.

Our investigations are therefore focused on the development of incipient colonies and on the technique of fostering.

The development of the incipient colony

Macrotermes alates were collected in the field (Kajiado area) and placed in plastic petri dishes containing steam-sterilized soil. The male-female pairs were checked at weekly intervals. Mortality was relatively high and similar in both sexes. It could be observed that survival is very much a function of having a healthy partner: many more pairs were found alive than was expected given the theory that survival is independant of the partner.

A general schedule of the incipient colony development of *Macrotermes* under laboratory conditions at 29°C is shown in Figure 1 (overleaf). This was obtained by summarizing the observations on 20 healthy colonies. We never observed spontaneous formation of fungus combs. Therefore we had to introduce small pieces of fungal material from a mature nest to the young colony as soon as the workers started foraging. These *Termitomyces* were generally accepted by the colonies, and were used as a starting point for their own fungus comb by adding pellets of fecal material. When fungus structures are present, the workers build a thin-walled spherical cell made from soil around the royal pair, including fungus, larvae and eggs.

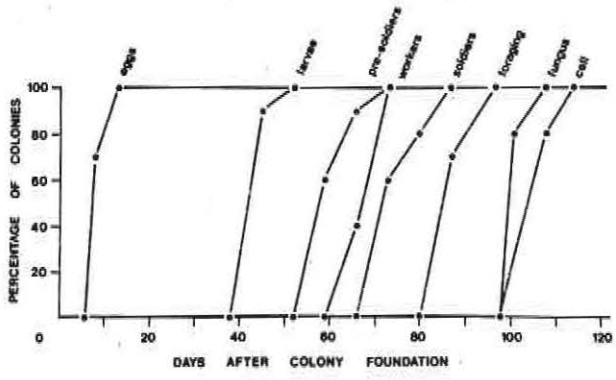

Figure 1. *Macrotermes*—incipient colony development

The effect of soil on *Macrotermes* incipient colonies

Three different soils were tested for their suitability for breeding *Macrotermes* incipient colonies. Two came from areas in Tsavo National Park, where *Macrotermes* mounds are frequent, the other soil was collected around ICIPE buildings on Chiromo Campus, Nairobi. Rearing termites was possible in all three types of soil. There were some significant differences in respect to survival, and the time when the first eggs, workers, pre-soldiers and foraging workers appeared. But on the whole no real advantages were found when the termites were brought up in Tsavo soil. It became evident, however, that for obtaining comparable results, it was very important to use soil from the same place.

The capacity of pre-soldier formation in incipient colonies

Every young *Macrotermes* colony produces one or two pre-solders about a week before the first pigmented workers appear. The pre-soldiers undergo their final moult and become pigmented minor soldiers a few days after the workers can be observed. The number of pre-soldiers and soldiers is an indicator of the size of the colony and ranges around 5% of the total number of individuals in the young colony.

In order to investigate the regulation of soldier formation an extraction-addition experiment was performed. Incipient colonies of the same age were divided into three groups, just before the first pre-soldiers would have appeared. They were checked every two or three days. All the pre-soldiers which eventually appeared in the donor colonies were removed and added to a receiver colony. The control colonies remained undisturbed. This experiment clearly demonstrated that incipient *Macrotermes* colonies are able to produce up to three times the number of pre-soldiers as compared to the control, within a very short time when these are extracted systematically. Receiver colonies themselves hardly produce any pre-soldiers. From our results therefore, it is clear that the mechanism of regulation on the colony level can be located somewhere around the second larval instar. It still cannot be decided whether it works by "re-programming" larvae, which otherwise would have become minor workers, or if some sensitive nestmates protect, or kill larvae which are predetermined to become soldiers.

Fostering of termite eggs

It is evident that eggs and larvae of *Macrotermes* require a great deal of attention to ensure normal development. It was possible to rear *Macrotermes* eggs successfully by using pairs of males or pairs of females of the species *Odontotermes badius*. In some cases complete development up to pigmented workers and soldiers could be observed. In general, however, the larvae disappear after their first or second moult. *Odontotermes* males are slightly better foster parents than pairs of *Odontotermes* females. Couples of *Odontotermes* (male and female) seem to be unwilling to accept foreign eggs—they bury them in a remote part of the box and continue caring exclusively for their own eggs.

Better results are obtained by intraspecific fostering. The results obtained so far indicate that with *Macrotermes* also, males are slightly more successful for rearing than females. This may be caused by the continuing egg production of the virgin foster-mothers. The caste composition of the offspring does not seem to be influenced by the sex of the foster parents and there is only a slight difference in instar duration of larvae brought up by two females or by two males. Larvae raised by males develop into slightly larger final instars. There are no morphological differences between eggs from an incipient colony and eggs from a mature colony in the field. But if given to foster-parents for breeding, field eggs give rise to larvae with longer instar duration and larger final instars. The caste composition is similar. There were slightly more soldiers derived from the incipient colony eggs but this difference is only significant at the 5% level.

The results obtained so far indicate that different mechanisms are involved in caste determination. Soldier development is regulated during larval development and is controlled by the presence or absence of pre-soldiers and soldiers in the colony. Nutritional factors (*Odontotermes*- or *Macrotermes*- foster-parents, males or females) slightly affect size and instar duration of the offspring, but are probably not important in caste differentiation. The sex-ratio (major workers/minor workers + pre-soldiers + soldiers) is most likely to be genetically controlled. Finally, we must expect plasmatic factors in the eggs which are responsible for the speed of development, instar duration and body size. These plasmatic factors are connected to the age and physiological condition of the mother.

Further studies on caste differentiation in *Macrotermes* near *subhyalinus*

B. M. Okot-Kotber

Caste differentiation has long been known to occur in both lower and higher termites. Polymorphism is evident, but our knowledge regarding this phenomenon is limited. It is known for certain now that caste differentiation in lower termites is determined by levels of juvenile hormones. As far as higher termites are concerned, we lack this information apart from fragmentary reports in the literature. The species we are dealing with belongs to higher termites. Investigations were therefore planned in an attempt to unravel the secret behind the mechanism of caste differentiation in our species. The present report summarizes the results obtained from a follow-up study of last year's experiments (ICIPE Annual Report, 1976).

Two main aspects of work were tackled during the year. First, morphometric work on the larval instars and polymorphism on field material was conducted as follows: the head capsule width, posterior tibia length, antennal length and the number of antennal segments of larvae and adult castes were determined. Secondly, histological investigations on corpora allata (CA), the glands that synthesis and secrete juvenile hormones (JH) were carried out.

The results have showed that the larvae could be distinguished into six groups, the sixth one being a little sclerotized. The adult castes were characterized into four groups—minor and major workers and minor and major soldiers (Table 1).

The larval instars of minor and major workers were then determined by the investigations on sexes. The six groups of larvae fall under two categories: males and females. Larvae in group 1 are a mixture of male and female individuals. Those in groups 2, 4 and 6 are all females and in groups 3 and 5 are all males. Minor workers are females and major workers are males. Logarithmic plots of tibia length against head capsule width of individuals in each group revealed that groups 2, 4 and 6 of larvae and minor workers lie within one curve (A) and those of groups 3 and 5 larvae and major workers lie within another curve (B) (Figure 1 overleaf). These observations allow us to postulate that from group 1 larvae, two types of larvae may emerge: the larger ones (males-group 3) which subsequently develop into major workers by moulting through group 5 larvae, and the smaller ones (females-group 2) which develop into minor workers and group 6 larvae through group 4.

The fate of group 6 larvae was established by rearing them in the laboratory using incipient colonies. It was shown that group 6 larvae are the precursors of major pre-soldiers. Other observations showed that minor pre-soldiers moult directly from some of the group 4 larvae.

Table 1. Characterization of larvae into groups and adults of a species related to *Macrotermes subhyalinus* depending on the morphometry carried out

Group	Development stage	Sample size	Head capsule width (mm)±S.D.	Posterior tibia length (mm)±S.D.	Tibia length/ head capsule width	Antennal length (mm)±S.D.	Number of antennal segments
1	1st Instar Larvae ♂ ♀	27	0.55±0.03	0.32±0.01	0.58±0.03	0.78±0.06	13
2	2nd Instar Larvae ♀	35	0.80±0.02	0.64±0.03	0.80±0.04	1.24±0.11	15
3	2nd Instar Larvae ♂	46	0.97±0.02	0.72±0.02	0.75±0.03	1.60±0.04	15
4	3rd Instar Larvae ♀	67	1.19±0.03	1.19±0.02	1.00±0.02	1.97±0.12	17
5	3rd Instar Larvae ♂	46	1.76±0.05	1.49±0.07	0.85±0.03	2.91±0.12	17
6	4th Instar Larvae ♀	28	1.80±0.01	2.02±0.06	1.12±0.03	2.80±0.08	18
—	Minor Workers ♀	38	1.64±0.08	1.70±0.09	1.04±0.04	2.33±0.13	18
—	Major Workers ♂	29	2.66±0.13	2.33±0.09	0.88±0.04	3.83±0.15	18
—	Minor Presoldiers ♀	23	1.75±0.10	1.99±0.05	1.14	—	17
—	Major Presoldiers ♀	45	3.11±0.36	2.79±0.16	0.90	3.86±0.23	17
—	Minor soldiers ♀	30	2.78±0.11	2.94±0.05	1.06	—	17
—	Major Soldiers ♀	30	4.55±0.17	3.77±0.11	0.83	—	17

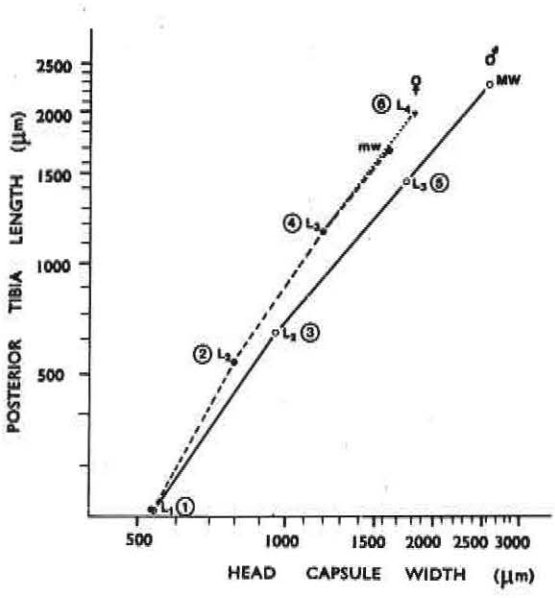

Figure 1. Logarithmic relationship between head capsule within and the posterior tibia length of larvae and workers of a species related to *Macrotermes subhyalinus*. Curve A represents the relationship among one category of larvae and minor workers and curve B represents the other and major workers

L_1, L_2, L_3, & L_4-Larval instars (1), (2), (3), (4), (5), & (6)-Larval group
mw-Minor workers
MW-Major workers

These results put together may permit us to propose a scheme of postembryonic developmental stages in our species of *Macrotermes* (Figure 2).

With all the instars known, it was possible to embark on the histological investigations in an attempt to understand the role of CA (JH) in the differentiation. Larvae from all the instars were processed histologically. In order to determine the activity of the endocrine glands, the sizes of CA and the number of nuclei within the maximum cross-sectional area of each gland, and the size of the nuclei and also of the prothoracic gland (PG) nuclei, were determined. The histological changes in the glands were noted. The results are summarized in Table 2.

A sexual dimorphism is expressed during the development of the larvae, male larvae having larger CA than females of the same instar. The maximum size is achieved during the third instar in both sexes. However, during and after pigmentation the size of the glands of minor and major workers decrease to a lower level than the third instars and they become of about the same size at this stage. The increase in CA size is most marked in the group 6 (fourth instar) larvae. This is true also for the CA nuclei size and cytoplasmic material, although the number of CA nuclei, as in the rest of the material, remains almost constant throughout the development. Major pre-soldiers also have enlarged glands. Nevertheless, they are smaller than those of the fourth instar larvae.

The changes in the relationship between CA nuclear and cytoplasmic cross-sectional areas in the various instars are illustrated in Figure 3.

It seems that during the larval development there is a more rapid increase in the amount of cytoplasmic material than there is in the size of the nuclei. This is most pronounced in the CA of fourth instar larvae followed by that of the pre-soldiers. It is apparent therefore that the activity is correlated more to the amount of cytoplasm than to the size of the nuclei.

Other interesting histological features observed in the cells of CA included packed nuclei in smaller glands and more dispersed nuclei in larger ones. Some CA have apparently vacuolated nuclei or cytoplasm which others do not have. It seems that vacuolation occurs following a period of high activity of the glands.

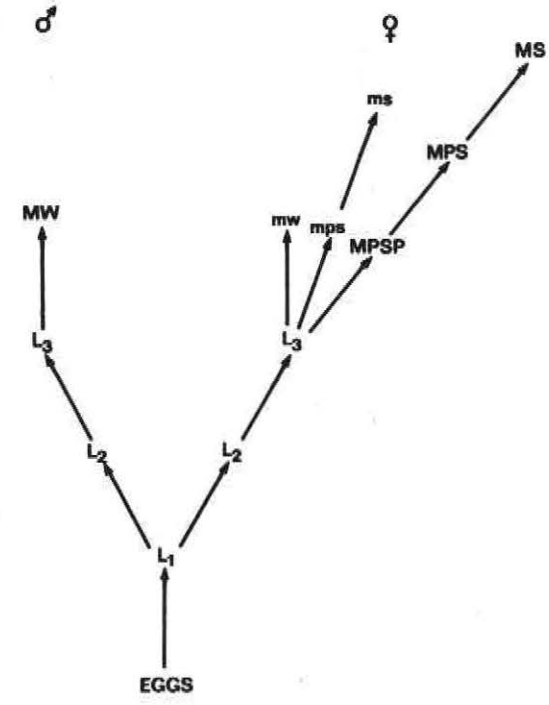

Figure 2. The possible shunts of post-embryonic development of neutor castes of a species related to *Macrotermes subhyalinus*

L_1, L_2, L_3 — Larval instars of workers
MW — Major workers
mw — Minor workers
mps — Minor presoldiers
ps — Minor soldiers
MPSP — Major presoldier precursors (fourth instar larvae)
MPS — Major presoldiers
MS — Major soldiers

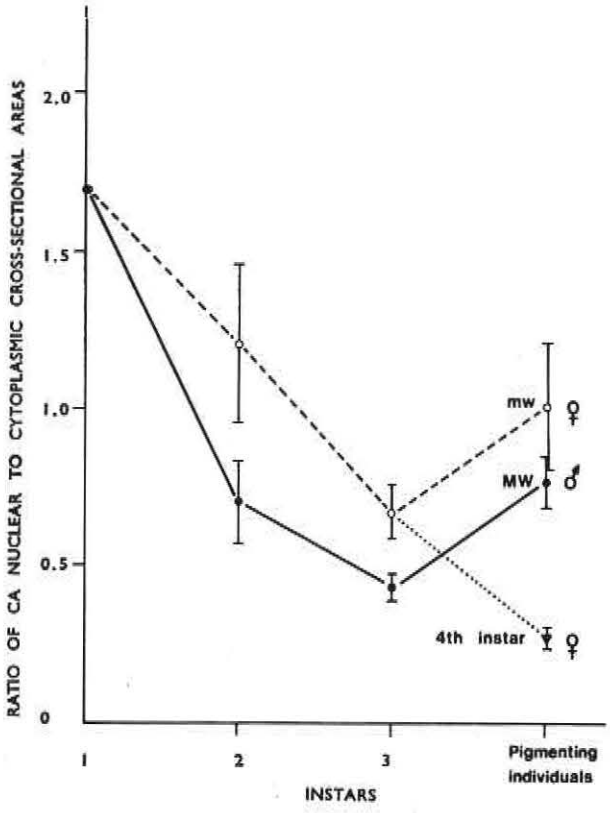

Figure 3. Changes in the relationship between the sizes of CA nuclei and the cytoplasm during development of larvae into neutor castes of a species related to *Macrotermes subhyalinus*. Vertical bars denote standard error of the mean
mw — Minor workers
MW — Major workers

Turning to the prothoracic glands one may find that they are within the head capsule of all the specimens studied. Some of the PG cells have elongated nuclei barely surrounded by any cytoplasm. This seems to be related to less activity of the glands since it is primarily observed in animals which have undergone a final moult. The oval-shaped nuclei of varying sizes surrounded by appreciable amounts of cytoplasmic material are observed in the PG of fourth instar larvae and of some individual larvae in their final stages of development. It is apparent that large PG are required where the most drastic change in size and shape has to occur during development.

From these findings there is an indication that JH may be playing a vital role in caste differentiation in *Macrotermes* as well. This is illustrated particularly by the observation that there is a relationship between enlargement of CA of fourth instar larvae and the soldier differentiation. Third instar larvae, both males and females, have much smaller glands and they moult into workers suggesting that for the development of a worker caste, relatively low amounts of JH may be required.

Experiments are underway using JH analogues to test further the role of JH on caste differentiation in higher termites, at least in our species of *Macrotermes*.

Table 2. Changes in the sizes of corpora, allata their nuclei, the prothoracic gland nuclei and the number of CA nuclei within the maximum cross-sectional area during larval development into workers and differentiation into soldiers of a species related to *Macrotermes subhyalinus*

Development stage	Sample size	Maximum cross-sectional area of C.A. (μm^2)±S.E.	Maximum number of nuclei in the cross-section of C.A.±S.E.	Maximum cross-sectional area of nuclei (μm^2)±S.E.	Area of nuclei/ area of cytoplasm ±S.E.	Diameter of PG nuclei (μm)±S.E.
1st Instar Larvae ♂ & ♀	30	460.65±25.57	12.40±0.31	20.05±0.53	1.70±0.26	5.69±0.21
2nd Instar Larvae ♀	28	572.16±27.09	13.64±0.35	19.46±0.20	1.20±0.25	5.98±0.21
2nd Instar Larvae ♂	24	749.23±34.20	13.62±0.58	19.63±0.00	0.71±0.13	7.12±0.34
3rd Instar Larvae ♀	18	760.22±36.97	14.35±0.51	20.53±0.86	0.67±0.09	6.37±0.70
3rd Instar Larvae ♂	24	879.70±44.59	12.76±0.33	19.16±0.46	0.43±0.04	7.71±0.46
4th Instar Older Larvae ♀	40	2864.46±110.13	12.57±0.35	43.38±1.43	0.27±0.03	13.95±0.43
Pigmenting Minor Workers ♀	12	628.49±17.23	14.86±0.78	20.36±0.96	1.05±0.20	5.76±0.38
Pigmenting Major Workers ♂	6	653.86±37.40	13.64±0.42	21.02±1.88	0.76±0.08	6.25±0.29

The influence of food stimuli on recruitment and food consumption in *Trinervitermes bettonianus*

G. W. Oloo

Previous studies on the foraging behaviour of the grass feeding termite, *Trinervitermes bettonianus*, indicated that the discovery of food stimulated recruitment. It was also found that at least two behaviourally distinguishable pheromone trails are involved in foraging activity—a "regular trail" used for orientation outside the nest and for maintaining contact with the colony; and a much more attractive "recruitment trail", laid by a food-finder, which is able to deviate termites from a "regular trail" towards a food source. Work described

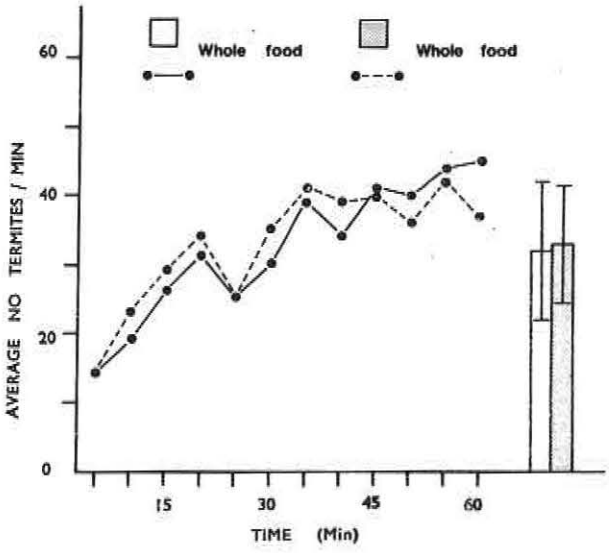

Figure 1. The influence of food taste on recruitment of *Trinervitermes bettonianus*

in this report examines the source of the recruitment stimulus in food, and the effects of food quality on the recruiting power of trails laid from it. The relationship between food quantity and foraging effort and the feeding preferences of the termite have also been studied. All the experiments were conducted with intact colonies dug from the field and maintained in the laboratory.

Food Quality

The influence of food odour was tested in a 2-way choice experiment using a T-shaped perspex tunnel fitted from either end with two glass tubes. The tubes were stuffed, respectively, with food and filter paper as the control and then fitted with wire gauze to allow for olfactory stimulation without direct contact with food. Food was presented at various distances by sliding the glass tubes in or out, and single starved workers were used as test termites.

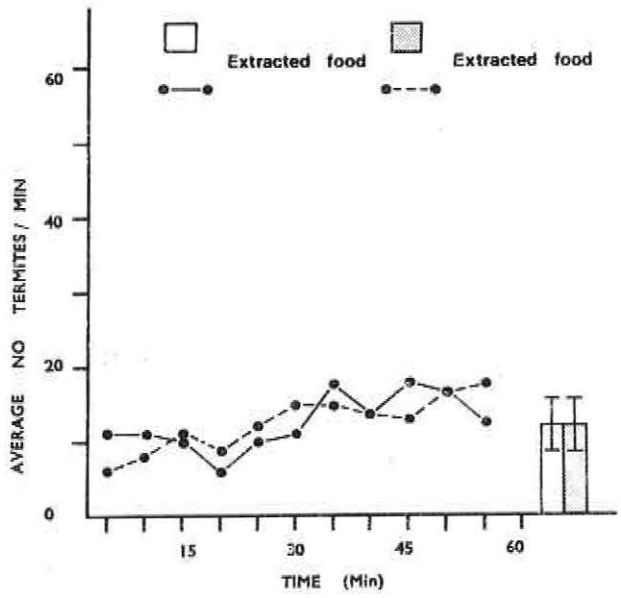

Figure 2. The influence of food taste on recruitment of *Trinervitermes bettonianus*

The response of foraging termites to phagostimulants was investigated by extracting normal food (ie. dry, dead grass litter usually fed upon in nature) with 80% Methanol for 3 days. The extracted grass was sun-dried and the extract used to impregnate filter paper cut into grass-sized pieces. Food quantities of 200mg each were used and the residues left after foraging were re-weighed.

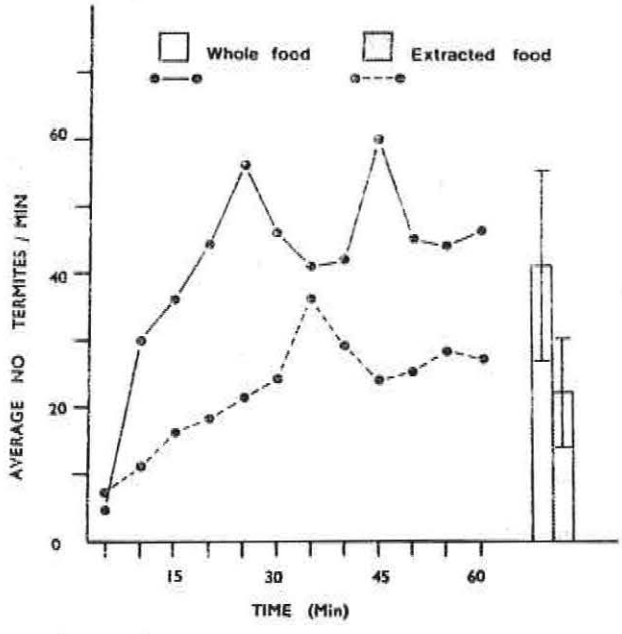

Figure 3. The influence of food taste on recruitment of *Trinervitermes bettonianus*. The difference between the means of termites offered whole food and extracted food was significant at P=0.001

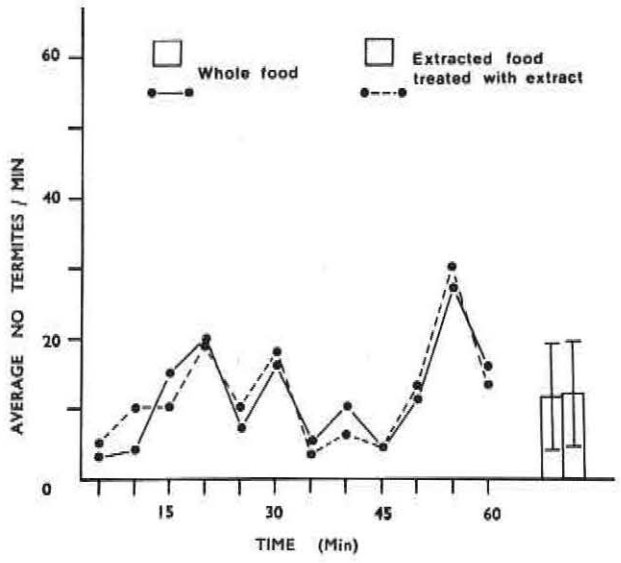

Figure 4. The influence of food taste on recruitment of *Trinervitermes bettonianus*

Foraging activity as estimated from traffic counts were compared by Student's '*t*'; the food quantities consumed recorded; and the strengths of trails laid to each food source compared by bioassay. In this experiment it was essential that the alternative choices of food be discovered almost simultaneously (or initial traffic to each source be comparable) and use of starved colonies avoided because of their non-selective feeding habit.

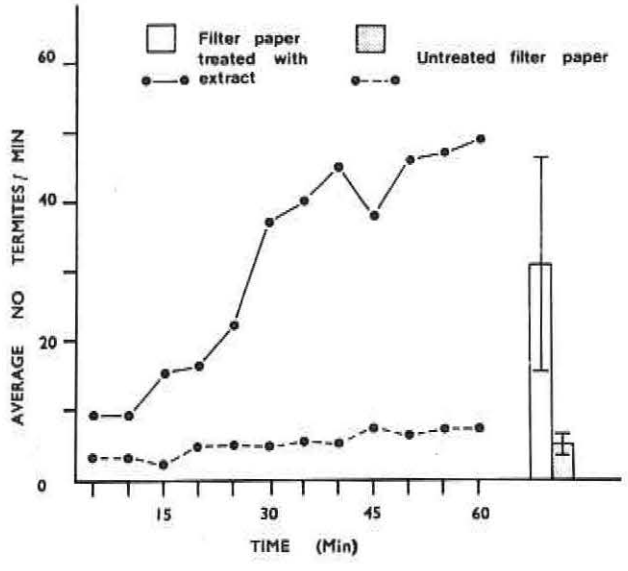

Figure 5. The influence of food taste on recruitment of *Trinervitermes bettonianus*. The difference between the means of termites offered filter papers treated with extract and untreated filter papers was significant at P=0.001

The potency of the food-oriented "recruitment trail" was determined by simultaneously offering normal food against extracted food, and food in one chamber against an empty feeding chamber in a 2-way choice situation. Filter paper with a pencil-drawn "Y" pattern was placed on the feeding bridge to collect the natural trail laid by foragers. The termites laying the trail were also recorded. The filter paper was then quickly removed, each arm of the "Y" was cut off so as to obtain equal strips bearing the trail, and the strips were cut into pieces and dropped into 200µl hexane to extract the trail pheromone. The extracts were assayed for potency with a 2-choice maze.

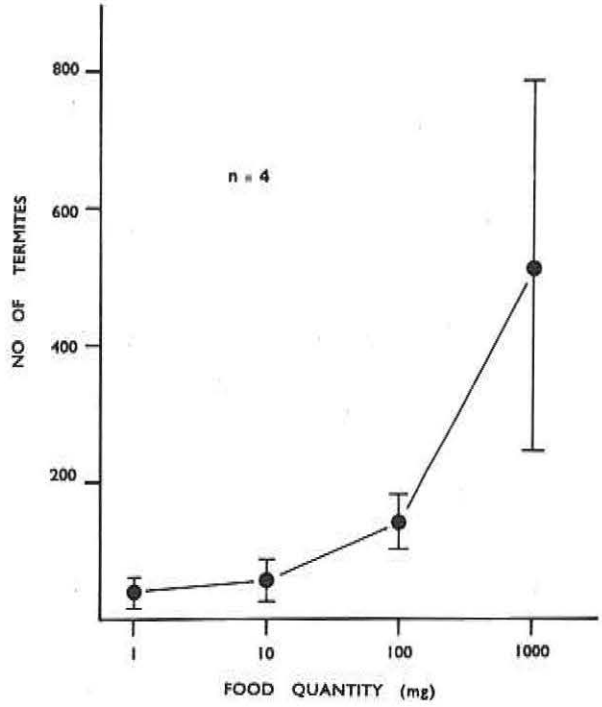

Figure 6. Relation between food quantity and foraging effort in *Trinervitermes bettonianus*

Results

The influence of volatile components of food (ie. food smell) was found to be negligible as no clearcut response could be obtained.

Comparative tests with sets of normal food (un-extracted whole) or extracted food alone, revealed no significant difference (Figure 1 and 2); but when presented with a choice between normal food and extracted food, the termites showed a strong preference for the former (P=0.001) (Figure 3). However, treating the extracted food with the food extract appeared to restore its attractiveness, comparable to that of normal food (Figure 4). Furthermore, recruitment to impregnated filter paper was significantly higher than to untreated filter paper (P=0.001) (Figure 5), although the treated paper compared unfavourably to normal food (P=0.05). These results indicate that food taste is an important stimulus for recruitment and consumption. In preli-

minary chemical analysis, reducing sugars in the polar fraction of the extract constituted about 2.3% of the total weight of food.

In the trail potency tests, the strength of the trail leading to normal food was found to be up to 10 times higher than that leading to extracted food.

Food Quantity

To find out the relationship between foraging effort and food quantity, food was presented in a feeding box in quantities of 1mg, 10mg, 100mg and 1000mg in an ascending order at intervals of 10 minutes. Each sample was presented to foragers for 10–15 minutes (ie. the time it took, on the average, to consume the smallest of the samples).

The results from termite counts in the foraging arena indicated that foraging effort was proportional to the quantity of food available (Figure 6).

Food preference

In order to investigate food preference, several grass species were collected from three different localities where the termite naturally occurs. The grasses were collected at flowering stage to minimize variation in quality due to age; the material was sun-dried; leaves were then cut into pieces 3cm long, weighed and fixed vertically into a perspex box—the samples being arranged in a randomized block design with 7 replications. The feeding box was then connected to the nest by a feeding bridge and after 6–8 hours of foraging, the grasses were weighed again to determine the quantities consumed.

In the analysis by F-test, it was found that the termites had a definite preference for certain grass species from a given area, but the order of preference among the preferred species seemed to vary (Table 1).

Conclusions

The finding that food quality is essential for stimulating trail pheromone production now provides a link in the chain of recruitment process. Thus, it appears that phagostimulants in food motivate a termite worker to lay a "recruitment trail" back to nest; the worker further alerts nestmates which then follow the food-finder's trail to the food source; they in turn become recruiters, if rewarded; and the repeated process develops into mass recruitment for normal food-gathering. A similar mode

Table 1. Food preference (quantity of food in mg consumed in 6 hours; food collected at flowering stage one year before the experiment)

0	I	II	III	IV	V	VI	VII	Total	Mean
A	14.3	1.0	10.0	19.3	14.9	16.5	15.8	91.8	13.1
B	4.1	4.3	5.1	6.6	7.6	11.0	12.8	51.5	7.4
C	12.0	12.6	8.4	5.5	10.2	20.6	8.6	77.9	11.1
D	0.6	5.8	3.0	8.6	6.0	4.5	13.4	41.9	6.0
E	11.1	14.5	3.6	7.0	4.8	18.1	21.4	80.5	11.5
F	10.0	11.7	9.4	2.3	15.1	13.9	11.3	73.7	10.5
G	18.4	10.1	15.2	5.0	15.8	5.0	4.5	74.0	10.6
H	4.9	5.5	7.2	5.5	5.3	5.3	3.4	37.1	5.3
I	0.0	4.6	0.0	4.2	4.7	4.5	4.6	22.6	3.2
J	1.5	4.6	4.1	5.0	4.9	2.8	6.5	29.4	4.2
K	9.6	18.8	19.5	17.0	18.6	5.5	23.0	112.0	16.0
Total	86.5	93.5	85.5	86.0	107.7	107.7	125.3	692.4	
Mean	7.9	8.5	7.8	7.8	9.8	9.8	11.4		

Rank: K A E C G F B D H J I

K—*Digitaria malinjiana*
A—*Loudentia kagerensis*
E—*Hyparrhenia papillipes*
C—*Aristida adoensis*
G—*Themeda triandra*
F—*Hyparrhenia hirta*
B—*Eragrostis lasiantha*
D—*Setaria sphacelata*
H—*Cymbopogon excavatus*
J—*Sporobolus pyramidalis*
I—*Panicum maximum*

of recruitment has been reported in ants. It is not known whether the observed functional differences between a "recruitment trail" and a "regular trail" are qualitative or quantitative, but the present study indicates that the former is several times more attractive than the latter. Thus, an established trail to good quality food (un-extracted) was found to be up to ten times stronger than that leading to poor quality food (extracted). The observed relationship between food quantity and foraging effort presumably provides a means of regulating energy utilization in foraging activity. The recorded food preferences of this termite species offers one possibility of determining whether the termite is competitive with livestock or game animals in the ecosystem.

Building behaviour in *Macrotermes*

O. Bruinsma

The behaviour of the termite *Macrotermes* near *subhyalinus* with respect to nest (re-) construction has been analysed for the past three years. The main results are summarized below.

In order to study building behaviour experimentally, it was necessary to devise several situations which are reproducible both in the field and under laboratory conditions.

Situation 1. Replacement royal cell construction. (a) Building behaviour of workers around an exposed physogastric queen of the same species will lead to the construction of an earthen vault which will cover the queen. This behaviour appears to be elicited and partly modulated by a pheromone released by the queen.

The royal "fat body" contains the source of this pheromone which is emitted via the permanently open spiracles. The concentration gradient of the pheromone may be used by the building workers as a cue for distance measurement. However, other mechanisms (eg. memory) could play a role.

(b) In addition to this queen building pheromone, the building workers employ various "tools" of a chemical and mechanical nature. First, the trail pheromone network around the queen is of prime importance for the stimulation of mass building activity and secondly, individual odour trails may function as a directional cue: workers with their load are able to follow the trails of their predecessors and make deposits at the same place.

During the cementing process, a secretion is released by the worker from the buccal cavity and added onto the soil bit. The bit acquires a behavioural dimension in the form of a pheromone (originating from the salivary gland) evaporating from the drying cemented soil bit. This pheromone motivates non-building workers to join in and orientates building workers towards an active construction site. In addition, tactile stimuli are very important for the precise location of a deposition site, especially in the beginning of construction activity.

Situation 2. Foraging gallery and pillar construction. The construction of foraging galleries appears to be modulated by trail pheromone gradients along and above the odour trail. Thus, as a consequence, a changed gradient distribution will be expressed in future structural elements. Evidence for this mechanism have been obtained from the following experiments: (1) manipulation of a natural odour trail by the addition of trail-active extracts, (2) manipulation of the number of passing (trail-laying) workers, (3) manipulation of the evaporation space of the odour trail.

The deposition and cementing of soil particles in a limited area leads to the erection of pillars of variable height, between 0.4–2.5cm. After reaching this height, building workers start adding soil pellets in a more horizontal plane. This change in the direction of building is probably initiated by trail pheromone gradients extending in the vertical plane formed by the evaporating trail network surrounding the pillar base.

In conclusion, we may say that a very important part of the "blue print" of nest structure is expressed in various pheromone distributions and the workers specific behavioural reactions to these distributions.

The role of *Macrotermes* in soils

M. A. Arshad

Investigations to study the effects of termite activities on soil characteristics in relation to their pedogenetic development was initiated in the year under report. For this purpose a number of open and closed mounds (described in detail in the ICIPE Annual Report, 1975) occurring side by side as well as soil profiles in the adjacent area were sampled. Soil samples were air-dried and ground to pass through a 2mm sieve for various analyses.

Preliminary data indicated that the soil reaction was slightly more acidic for the soil in open mound than either the closed mound or the surrounding soil, although differences were small. Percentages of organic carbon of the mounds were much lower than the surface horizon of the adjacent soil, but decreased markedly below the mound (the sub-soil). Within the soil samples of the mounds the organic carbon increased from the outer casings towards the royal cell and then decreased with the depth of the mound. This is expected because of higher biological activity surrounding the royal cell. Water holding capacities for various chambers of both types of mounds were much higher as compared to the adjacent soil. This is attributable to the higher fine clay content of the mound soils than the adjacent soil.

The values ranged from 28% for surface soil to 55% for royal cell of open mound. The fresh sample of fungus comb exhibited the maximum water holding capacity of 95%. The fungus material thus appears to play an important part in maintaining moisture status and regulating relative humidity inside the mound structure.

The study concerning the effects of termite activities on soil erosion is still in the initial stages. A suitable site for construction of runoff plots to measure soil loss as a result of termite activities has been selected. This particular experimental area, located near Kajiado, will also be used to monitor changes in soil productivity, vegetation patterns and the dynamic soil processes.

PROGRESS AT KAJIADO FIELD STATION

Since July 1977 the Field Station staff has been increased by the addition of a UNEP-sponsored trainee, Mrs. T. C. Aloo, who is working on the consumption by termites and other insects of cattle (and other) dung. As a result of the acute housing shortage in Kajiado, living accommodation for Mrs. Aloo has had to be provided on the Field Station premises. The extra temporary laboratory (provision of which was regarded by the scientists at the station as an essential prerequisite to any increase in staff) was not provided in time to avert considerable inconvenience, and at the time of writing (end of December 1977) is still not completed. This has caused disruption to the existing work programme through acute shortage of working space.

Further problems were encountered as a result of a break in the water pipeline from Ngong which left most of Kajiado virtually without water for six weeks in September–October. The pipeline is now working again but to avoid such problems in the future a static water tank has been ordered, and a tank trailer purchased for emergency use.

Difficulties over housing are anticipated in 1978, since the Maasai Technical School, from which both the resident scientists rent houses, is taking in more pupils and staff and is likely to need the houses for its own use. Provision of permanent housing for ICIPE staff in Kajiado should be given a high priority, if it is intended that the field station be kept running at its present level.

LIVESTOCK TICK RESEARCH

Visiting Directors of Research
Professor T. O. Browning (1970) Ecology
Professor R. Galun (1970) Physiology

Project Leader
Dr. M. P. Cunningham (1977)

Research Staff
Mr. A. Bwire (1975) Technical Assistant
Mr. J. W. Chiera (1976) Research Assistant
Mr. G. M. Hindi (1974) Laboratory Assistant
Mrs. C. K. A. Mango (1971) Scientific Officer
Mr. J. G. Mugane (1973) Laboratory Assistant
Mr. J. N. Ndungu (1973) Laboratory Assistant
Dr. R. M. Newson (1974) Senior Research Scientist
Dr. F. D. Obenchain (1976) Research Scientist
Mr. R. Ojowa (1972) Junior Technician
Mr. D. K. Punyua (1973) Associate Scientific Officer
Mr. K. C. Wainaina (1974) Laboratory Assistant

Collaborators
Mr. K. R. Binnington, CSIRO, Australia
Mr. T. S. Dhadialla, Reproductive Physiology
Dr. T. Dolan, VRO, Kenya
Dr. A. Maradufu, Chemistry
Mr. A. O. Mongi, Tanzania Veterinary Department, ICIPE Fellow
Mr. L. Moreka, Technician
Dr. A. S. Young, VRO, Kenya

ECOLOGY

Studies on experimental *Rhipicephalus appendiculatus* populations and East Coast fever transmission

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

In 1973 a population of *Rhipicephalus appendiculatus* infected with *Theileria parva*, the causative organism of East Coast fever in cattle, was set up in co-operation with the former East African Veterinary Research Organization at Muguga. Its development and use, described in the 1974–76 ICIPE Annual Reports, has continued throughout 1977, under the Organization's present Kenyan status.

The paddock is divided in the ratio of 1(A): 2(B). Regular collections of larval, nymphal and adult ticks are made from the vegetation in both parts, in order to monitor changes in the tick populations, by drawing a cloth over the grass.

Effect on ECF infectivity of allowing ticks to feed only on immune hosts, or on no hosts

In order to simulate an ECF vaccination campaign, and to study the change in infectivity that would occur in the vector population when it could feed only on immune hosts, six cattle immune to *T. parva* were kept in part B from November 1975–September 1976. During the same period six pairs of susceptible cattle were exposed in the paddock for periods of 6 days, then removed to quarantine for observation, before they could return any fed ticks to the population on the pasture. The first pair died of ECF, but although the tick load had increased threefold the sixth pair showed only mild disease reactions (possibly due to non-pathogenic theilerial species), and remained susceptible on challenge with the original strain of *T. parva* that had been used to infect the paddock. Meanwhile similar pairs of cattle introduced into part A and left there consistently, contracted acute ECF and died, thereby confirming the continued transmission of the disease under the prevailing field conditions.

During September 1976–September 1977 no cattle were grazed in either part of the paddock, and the monitoring showed a sudden extinction of the larval part of the tick populations in May–June 1977, a more gradual decline in the nymphs, and the continued survival of the adults even after 15 months of starvation. There was a marked increase in numbers collected during and after the 1977 long rains (April–June, see Figure 1 overleaf).

In September, and again in December 1977, three susceptible cattle were exposed for 6 days in part B and one in part A. They were all removed to quarantine as before. Their tick loads on day 6 were from one-third to two-thirds as high as those in September 1976, but in each case the animal from part A died of acute ECF, thus confirming the persistence of *T. parva* in the ticks over an interval of up to 15 months. As predicted, the

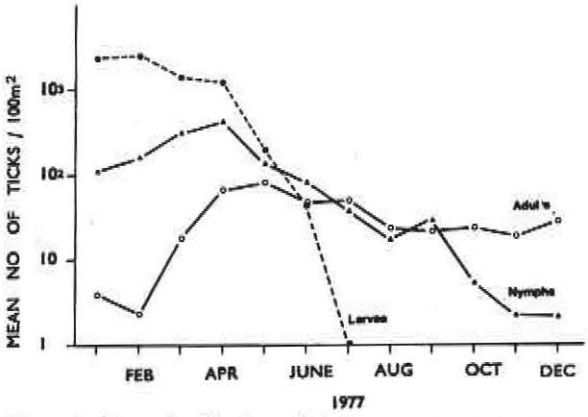

Figure 1. Ground collections of ticks in part B of experimental tick infested paddock; results per 100m² sampled, expressed as $\log_{10}$ (mean +1)

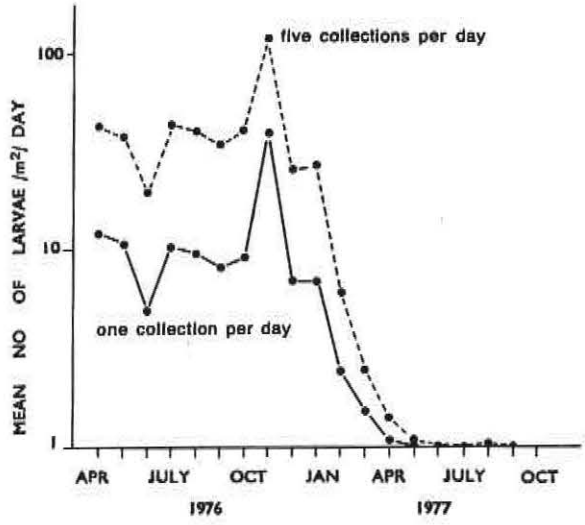

Figure 2. Collections of larvae from a group of nine isolated 1m² quadrats in part B of the experimental tick infested paddocks, mean number of ticks per m² expressed as $\log_{10}$ (mean + 1)

cattle that had been exposed in part B remained healthy and were subsequently shown to be fully susceptible to *T. parva* on challenge. The paradox of markedly increased numbers of adult ticks on the vegetation in 1977, but reduced numbers attached to the cattle with no possible recruitment, is explicable if the surviving and hungry ticks became more active as they aged.

Daily collections of ticks from isolated quadrats

Collections from a set of nine isolated 1m² quadrats were each continued in parts A and B throughout 1977 (Table 1), as described in the 1976 ICIPE Annual Report. Larvae, nymphs and adults were collected five times per week. After more than one year of abundance the larvae disappeared abruptly in March-April 1977. As in the rest of the paddock, the catch of adults increased again in the second and third quarters with the long rains. The yields of adults in 1977 were only a little less than in 1976, though by now all were 1–2 years old.

Similarly, collections were continued from another set of nine isolated quadrats set up in April 1976 in part B, in which the collecting effort for larvae and nymphs on four of the 1m² plots was five times more than on the remaining five plots. The overall yields per m² under the more intensive collecting were in the ratio of 4.5:1 for larvae and 4.2:1 for nymphs. The last larva was found in August and the last nymph in October. The results for larvae are shown in Figure 2; the pattern for nymphs was similar. The conclusions to be drawn from this study are that our collecting method picks up only a small proportion of the ticks present on the vegetation at any moment, since our data show very little reduction in yield for successive samples each day. There is also no evidence of marked stimulation in activity as a result of the collecting techniques, unless the two processes cancel one another out. Although the immature ticks move up and down the vegetation, it appears therefore that the actual transfer of these ticks to the collecting cloth, and presumably also to a live host, is passive. It is notable that even over the period of 555 days that was needed to collect all the available larvae and nymphs, the parallel relationship of the numbers taken under the two collecting regimes was maintained (for larvae see Figure 2) and the five times daily collecting did not appear to exhaust the supply of ticks any more quickly. It follows therefore that even the mean total densities of 1,220 per nymphs per m² and 8,797 larvae per m² obtained under the five times per day collecting were under-estimates of the true density at the start of the experiment. The abrupt extinction of the larval component of the population, though occurring 2–3 months earlier than in the rest of the paddock (see Figure 1), was nevertheless comparable, and suggests the sudden depletion of a resource, such as food reserves in the body.

Table 1. Ticks collected on two sets of isolated quadrats each consisting of 9 1m² plots in parts A and B of the tick-infested paddock

	A			B		
	adults	nymphs	larvae	adults	nymphs	larvae
January–December 1976	376	1825	19405	315	1266	17345
January–March 1977	47	296	2048	30	229	1319
April–June	162	75	37	110	32	18
July–September	105	43	10	73	0	0
October–December	35	3	0	17	0	0
Total	725	2242	21500	545	1527	18682
Yield/m;	80.6	249.1	2388.9	60.6	169.7	2075.8

Reproduction and survival of *Rhipicephalus appendiculatus* at different host stocking densities

R. M. Newson and J. W. Chiera

An experiment was started in June 1976, and details of its establishment and the early results were given in the 1976 ICIPE Annual Report. The development to five tick populations is being followed at three different host stocking densities by grazing a single steer each in two plots of 1,000m², two plots of 4,000m² and one plot of 12,000² in double-fenced paddocks at the Kenya Veterinary Research Organization at Muguga. The number of ticks on the cattle and on the vegetation, as well as the health of the cattle and the state of the vegetation are being closely monitored.

By October 1976, the cattle in the smallest plots had reduced the grass to a height of 4cm and they kept it at that level until August 1977. For most of this period it was necessary to augment their diet with hay and it was obvious that the situation was inherently unstable. The cattle were removed in August 1977, to allow the grass to recover, but monitoring of the tick population continued. On the other three plots the grass remained 20–25cm high until the 1977 rainy season, when it increased to 30–40cm. The grazing pressure only reduced the grass height by 5–10cm compared with an ungrazed control plot, so that there was still a thick protective sward, whereas on the smallest plots the very heavy grazing left little ground protection for the ticks. The rainfall in 1977 at the study area was 50% higher than average, which will have favoured survival of all stages of the tick on the ground.

The changes in the tick populations are best shown in the monthly estimates of total adults on the hosts (Table 2). It should be noted that populations No. 1 and No. 3 have shown parallel changes but that No. 3 has been consistently higher than No. 1 by a factor of 3 to a factor of 10 or more. Nos. 4 and 5 have been much closer together without consistent differences. The smallest plots each showed one distinct peak and two pronounced troughs, whilst the intermediate sized plots had peaks one month later and then decreased slowly. The largest plot has shown the least variation, with a gradual build-up and subsequent gentle decrease.

The results of the ground collections confirm the broad picture derived from the cattle. The first adults to be produced from the introduced immatures bred in 1976 and gave rise to successive cohorts of larvae, nymphs and more adults (the latter representing the peaks discussed above). During the second half of 1977 a second generation of larvae reached its highest numbers on all plots in October, then decreased sharply. Nymphs were still mainly members of the cohort that appeared early in the year, but began to increase irregularly as the second generation of larvae fed and moulted.

The conclusions so far are that the high chances of host-tick contact on the smallest plots leads to rapid changes in the tick populations. In contrast, on the largest plot the tick population appears to be already approaching stability, though at an intermediate level of numbers on the host, but with a fairly numerous, widely dispersed component on the ground acting as a buffer.

Table 2. Estimated total adult *Rhipicephalus appendiculatus* on the cattle from each plot, based on half-body collections (plot 2 was an unstocked control of 1,000m²). The peak: trough ratio (a) is from the lowest value preceding the highest value; (b) is from the lowest value following the peak

Plot No.		1	3	4	5	6
Plot Size		1,000m₂	1,000m²	4,000m²	4,000m²	12,000m²
Sept. 1976		106	390	276	132	146
Oct.		70	242	92	278	306
Nov.		36	129	93	277	268
Dec.		13	45	175	605	428
Jan. 1977		20	58	320	522	384
Feb.		160	1495	276	276	289
Mar.		382	3880	726	861	385
Apr.		361	3272	2849	4046	647
May		158	1489	2844	3367	885
June		32	429	1296	1346	576
July		13	147	397	744	295
Aug.		24	211	262	276	301
Sept.		—	—	164	210	252
Oct.		—	—	178	—	227
Nov.		—	—	257	151	351
Dec.		—	—	282	120	233
Peak: trough	(a)	29.4	86.2	31.0	30.7	6.1
	(b)	29.4	26.4	17.4	33.7	3.9

Anti-tick properties of molasses grass *Melinis minutiflora*

J. W. Chiera, R. M. Newson and A. Maradufu

Molasses, or gordura, grass *Melinis minutiflora* is an African species widely cultivated in the tropics for fodder. It is known to have strong anti-tick effects. The sheaths of the leaves are densely covered with fine hairs which exude a sticky secretion with a characteristic smell. We are investigating to see if the grass has any chemical component which might be exploited in tick control.

In the first experiment 20cm lengths of stem with leaf sheath of *M. minutiflora* were offered separately to larvae, nymphs and adults of *R. appendiculatus*, larvae of *Boophilus decoloratus* and nymphs of *Amblyomma variegatum* for them to climb. *Setaria sphacelata*, a common pasture species, was used as a control. Each test was replicated three times. All the ticks climbed the controls, generally to the top, but only a few *A. varigetatum* nymphs managed to ascend the *M. minutiflora* for even a short distance. All the other ticks were repelled.

The sticky secretion was found to dissolve readily in acetone, leaving the hairs intact, whilst air-drying alone eliminated the stickiness but not the smell. The next experiment was therefore to compare the climbing of *R. appendiculatus* larvae when offered (a) fresh (b) dried and (c) air-dried then acetone-washed specimens of *M. minutiflora* and *S. sphacelata*. The larvae readily climbed all the control material, but again avoided the fresh molasses grass, whilst a few climbed a short way up the dry grass. The acetone-washed grass was climbed just as readily as the control, so it was apparent that the anti-tick properties reside in the secretion, and not in the physical character of the hairs themselves.

Bulk samples of molasses grass were washed in five solvents, the extract solutions were evaporated to dryness and then re-dissolved (Table 3). Preference tests were done using *R. appendiculatus* larvae offered equal-sized pieces of filter paper on which samples of the solutions had been allowed to dry, against blank solvent controls. The tests were run in ventilated petri dishes and they were then frozen at −10°C to immobilize the larvae for counting. The results of six replicates were summed (Table 3). It is apparent that the petrol-ether and benzene-methanol extractions exerted the greatest repellency and work is proceeding to extract and analyse the active component.

Table 3. Responses of *Rhipicephalus appendiculatus* larvae to extracts of *Melinis minutiflora* in five solvents compared with blank solvent controls. Results of six replicates summed, C=control; E=experimental

First solvent	Second solvent		Total no. of ticks	Percentage distribution
Water	Water	C	840	69.8
		E	363	30.2
Methanol-acetone	Water	C	958	70.2
		E	406	29.8
Chloroform-acetone	Acetone	C	914	68.4
		E	423	31.6
Petrol-ether	Acetone	C	1537	98.4
		E	25	1.6
Benzene-methanol	Acetone	C	1504	92.6
		E	120	7.4

PHYSIOLOGY

Attraction of unfed females to attached males in Kenyan *Amblyomma* species

F. D. Obenchain, R. M. Newson and J. W. Chiera

In 1976 the responses of males and females of two *Amblyomma* species common in Kenya, *A. variegatum* and *A. gemma*, were tested against ether extracts of 10 day fed male *Amblyomma hebraeum*. After that period of pre-feeding, the males of this latter species are known to produce an extractable pheromone which induces unfed nymphs as well as adult males and females to attach in clusters around the feeding male. Neither unfed males nor females of the Kenyan *Amblyomma* species responded to the pheromone extracts of the *A. hebraeum* males, although the same extract was subsequently shown to be active against *A. hebraeum* females. While the males of the North American species *Amblyomma maculatum* are also known to produce an aggregation-attachment pheromone, the females of that species do not respond to the ether extracts of *A. hebraeum* males according to Rechav (personal communication), and he proposes that this pheromone system of *Amblyomma* ticks may show considerable species specificity.

Table 1 summarizes the results of a series of experiments which were initiated in 1976 to investigate the aggregation-attachment pheromone mechanisms of *A. variegatum* and *A. gemma*. These experiments utilized a bioassay in which groups of males were pre-fed for 7–8 days in cloth bags on the scrota of three bulls. At the end of that period all but 1 of the feeding male ticks were removed, the scrota were washed and the scrotal bag was replaced. On day 10 of feeding, 30 unfed ticks (males or females, according to the experiment) were introduced into each bag. Thirty unfed ticks were also introduced into scrotal bags on two control bulls with no pre-fed males present. The percentage attachment response and degree of clustering within 2.5cm of a 10 day fed male were recorded after 24 hours for experimental and control groups. In these experiments the attachment rates of unfed males of both species were unaffected by the presence of 10 day fed males and there was no apparent clustering of the newly attached males around the 10 day fed males. Females of both *A. variegatum* and *A. gemma*, however, showed a low attachment rate of about 10% in the absence of pre-fed males. When 10 day fed males were present the females of both species showed a highly significantly increased attachment rate of about 70% and displayed a high degree of clustering around the pre-fed male. Preliminary experiments showed that ether extracts of the 10 day fed males also elicited aggregation responses in females of the respective species.

Data from an experiment on the species specificity of this pheromone mechanism are summarized in Table 2. Two groups of males of each of the Kenyan *Amblyomma* species and one mixed species group were pre-fed for 7 days on the scrota of 5 bulls. After 7 days the numbers of feeding males were reduced to two per scrotum; bulls Nos. 1 and 4 had *A. gemma* males, bulls Nos. 2 and 3 had *A. variegatum* males, and bull No. 5 had one male of each species. On day 10 of male feeding groups of 30 unfed *A. gemma* females were placed in the scrotal bags of bulls Nos. 1 and 3. Similar groups of *A. variegatum* females were placed on bulls Nos. 2 and 4, while 30 females of each species were placed in the scrotal bag on bull No. 5. The attachment responses of the *Amblyomma* females was on the order of 80–90% in the presence of 10 day fed males of the same species, but when males of the reciprocal species were present the attachment response dropped to 50–60%. While these results seem to show some species specificity, the attachment responses in the reciprocal exposures are still significantly greater than the 10%

Table 1. Attachment parameters for two East African *Amblyomma* species

Sex Tested	Sex Present	% Attached *A. variegatum*	% Attached *A. gemma*
	None	95.71± 2.97	96.25± 2.63
$Male_{uf}$	$Male_{10df}$	90.00±10.00	
	None	8.82± 4.17	12.5
$Female_{uf}$	$Female_{6df}$	10.00± 3.33	
	$Male_{10df}$	69.80± 9.18	71.68± 9.80
% Clustered Around $Male_{10df}$		63.26± 9.86	68.35±12.22

Table 2. Inter- and intraspecific attachment responses of unfed females to 10 day fed males in two East African *Amblyomma* species

Female Sp.	Male Sp.	% Clustered	% Attached
A. gemma	*A. gemma*	90.0	90.0
A. variegatum	*A. variegatum*	68.9	82.8
A. gemma	*A. variegatum*	50.0	50.0
A. variegatum	*A. gemma*	58.6	58.6
	A. gemma	44.4	
A. gemma	*A. variegatum*	33.3	100.0
	A. variegatum	20.0	
A. variegatum	*A. gemma*	80.0	100.0

rate observed in the absence of any pre-fed males (Table 1). When unfed females of both species were placed together in a scrotal bag with one 10 day fed male of each species all of the females had attached within 24 hours. Approximately 45% of the *A. gemma* females were clustered around the *A. gemma* male, whereas 33% had clustered around the *A. variegatum* male. By way of contrast, 80% of the *A. variegatum* females were clustered around the *A. gemma* male and only 20% were clustered around the male of their own species. If the results of this preliminary experiment should prove to be repeatable they indicate that the aggregation-attachment pheromone mechanisms of Kenyan *Amblyomma* ticks have a low degree of species specificity in the context of the bovine scrotal-bag bioassay. While species non-specificity might add to the theoretical potential of *Amblyomma* pheromones in a programme of integrated tick control, basic studies on the chemical nature and environmental persistence of the pheromone(s), discovery of their sites of synthesis and release and detailed studies on the behaviour of the ticks have not been completed. Until the data from such studies have been obtained it is not possible to assess the feasibility of using this pheromone mechanism for the control of *Amblyomma* ticks.

Moulting hormone activity in the nymphal instars of the tick *Ornithodoros moubata*

C. K. A. Mango and L. Moreka

Ticks, like insects, develop and grow through intermittent replacement of their intergument. In insects, ecdysone is responsible for moulting, while in ticks only indirect evidence of the role of ecdysone exist. *Musca* bioassay is used to test the presence of ecdysone in insects.

The *Musca* bioassay was used to assess changes in the titre of moulting activity during the development of the fifth instar nymphs of *Ornithodoros moubata*. Fifth instar nymphs were artificially fed through membrane on pig blood and were then divided into 14 groups of 50 nymphs each. Starting on day 7 and continuing to day 14 after feeding, tick hamolymph was collected daily from the respective tick groups and extracted in 1ml of 10% ethanol.

Fourth and third instar nymphs were also extracted in methanol on 10th and 9th day respectively after feeding and the extracts were pooled together. Eight fractions were obtained from the pooled extract by gas liquid chromatography. These were assayed and the active ones identified and purified further.

The results show two peaks of activity in the extracts of day 7 to 14 after feeding. The first one is small and is reached on the 4th day and the second one which is quite steep is reached on the 9th day after feeding. Then there is a sharp drop in activity (Figure 1).

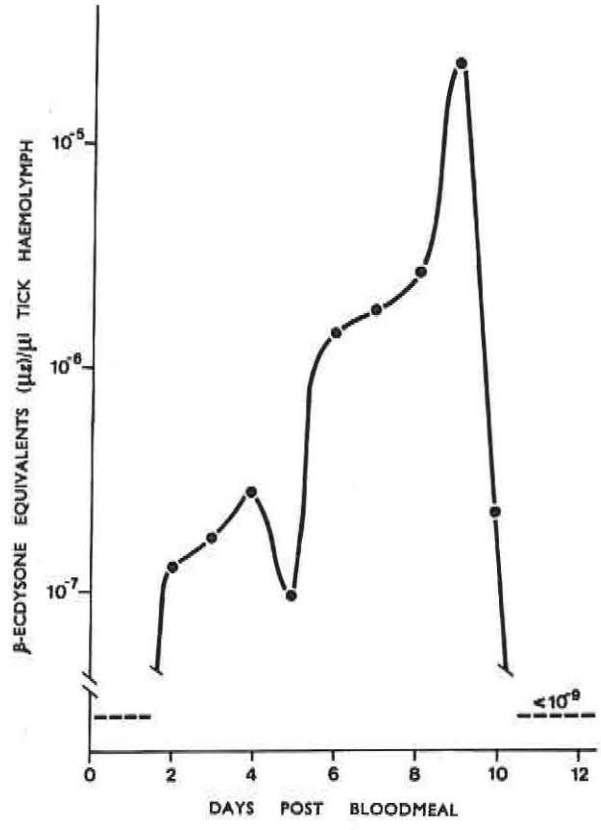

Figure 1. Relative moulting activity in the haemolymph of fifth instar nymphs of *Ornithodoros moubata* as determined in the *Musca* bioassay and expressed as µg equivalents beta-ecdysone. Values on days 0, 1, 11 and 12 were less than 1×10^{-9}

Among the eight fractions, one was found to be most active, giving about 58% activity. This fraction has been further purified and broken into 17 different fractions which are in the process of being assayed.

It can be concluded therefore, that tick nymphs during their development, possess a moulting hormone-like substance which shows moulting activity in *Musca*. In the fifth instar nymphs, the titre reaches the highest peak on the 9th day after feeding.

Studies on the feeding behaviour of female *Rhipicephalus appendiculatus*

F. D. Obenchain and T. S. Dhadialla

Rhipicephalus appendiculatus ticks which are infected with *Theileria parva* only become effective vectors after a certain period or amount of blood feeding. Similarly, the ability of ticks to re-attach after removal from a former host decreases with increased feeding, whereas the ovipositional ability increases with the degree of engorgement. The biological significance of these observations is clear and straightforward, but previous quantitative studies have suffered from aberations in the correlation between these parameters

and tick engorgement weights. Obenchain, Leahy and Oliver (in press) worked with the North American tick, *Dermacentor variabilis*, and devised a relative engorgement scale which is based on the geometric relationships between the tick scutal index (scutal width at the eyes × length in the midline) and tick weight. As derived from those relationships, the relative engorgement state of a tick can be expressed at any time before or during feeding as the ratio of the cube root of tick weight (mg) to the square root of the scutal index (mm^2) ($RES = W\frac{1}{3} \div SI\frac{1}{2}$). Preliminary experiments have shown that the same geometric relationships between tick weight and the scutal index which were found in *D. variabilis* also exist in *R. appendiculatus*. Accordingly, the following experiments used *RES* ratios as a tool for the study of engorgement behaviour in female *R. appendiculatus*.

The mean *RES* ratio for the unfed *R. appendiculatus* females used in these studies was computed as 1.029± .005, while normally mated and "fully engorged" females had a mean *RES* ratio of 5.351 ± .737. The threshold for the development of ovipositional ability was found to be at *RES* = 2.350. These findings imply that an average sized unfed female *R. appendiculatus*, weighing 2.66mg, could be expected to develop the potential for egg development and oviposition after reaching only 31.65mg, but that it would weigh about 375mg when fully engorged.

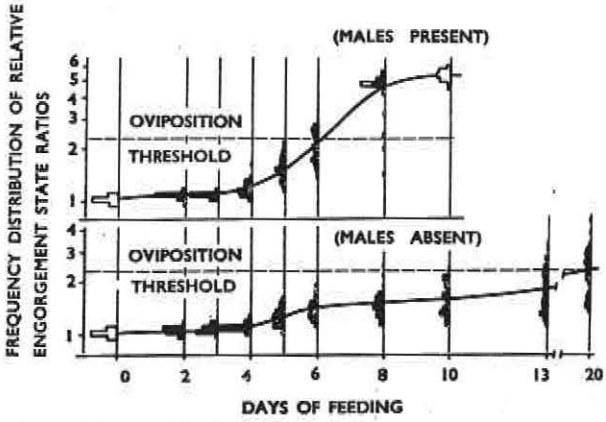

Figure 1. Effects of the presence or absence of male ticks on the frequency distribution of relative engorgement state ratios of female *Rhipicephalus appendiculatus* during the course of feeding on rabbit ears

- □ unfed female ticks
- ■ feeding female ticks
- ▨ replete female ticks

Figure 1 shows the changing distribution with time of *RES* ratios in two groups of feeding females. Females feeding with males show an accelerated rate of feeding on days 5 through 10, by which time all have engorged to repletion and fallen from the host. These data illustrate the previously well-known importance of tick mating for the promotion of full engorgement. By contrast, unmated females feed at a comparatively slower rate after day 5. Although these females do not willingly detach from the host, increasing numbers of females reach *RES* values beyond the threshold for ovipositional

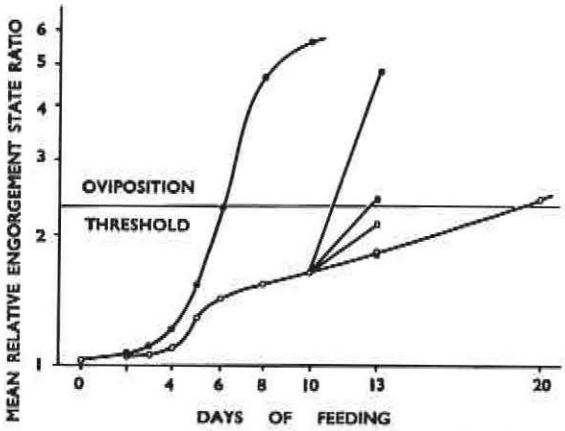

Figure 2. Effects of the presence or absence of males and of experimental treatments administered on the 10th day of feeding in the absence of males on the daily mean relative engorgement state ratios of female *Rhipicephalus appendiculatus*

- ● mated or males present
- o unmated, males absent
- ■ cyclic AMP injected
- ▫ cholera toxin injected
- ▲ water injected

ability by days 13 through 20. It was also established that unmated females of *R. appendiculatus* with *RES* values beyond the ovipositional threshold of 2.350 will develop and lay eggs, although these eggs do not hatch.

Figure 2 summarizes the results of the previous experiments and shows the results of a preliminary investigation on the effects of cyclic AMP on the promotion of rapid engorgement behaviour in unmated female ticks. On day 10 of feeding 4 groups (35 to 45 per group) of unmated ticks were submitted to experimental treatments, while a fifth group was used as a control. Group 1 females were mated and group 2 females were injected with 2µl of tick saline, while females in groups 3 and 4 were injected with 2µl of 2, 6 dibutryl cyclic AMP (25µg/µl) and cholera toxin (25µg/µl), respectively. Cyclic AMP levels in tick salivary glands are known to rise during the post-mating period of female rapid engorgement behaviour. The purpose of injecting the 2, 6 dibutryl cyclic AMP and the cholera toxin (a known stimulant of enzyme systems which generate cyclic AMPs) was to see if such treatments would mimic the effects of mating on the promotion of rapid feeding. Ticks in all five experimental groups were removed from their rabbit hosts on day 13 of feeding (3 days post treatment) and their *RES* values were determined. While the *RES* levels of cAMP and cholera toxin injected ticks were significantly elevated over the untreated control, they did not promote feeding to the degree observed in the mated females. Still these results suggest that the mechanism which switches females into rapid feeding behaviour may involve the promotion of high levels of cAMP in the salivary glands.

The use of *RES* ratios seems to be appropriate for studies on the feeding behaviour of *R. appendiculatus* and we propose to use it further in preliminary investigations of host resistance to tick feeding.

TSETSE RESEARCH

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Trypanosoma brucei: from the tsetse fly gut into an artificial medium: alive, infective, and lethal

T. R. Odhiambo and M. B. A. Nyindo

Trypanosoma brucei, EATRO 999, was grown from the mid-gut of the tsetse fly, *Glossina morsitans*. Freshly deposited larvae were placed in a clean sterile vessel and after pupation they were surface-sterilized in a solution containing mercuric chloride. They were placed on sterile sand in bottles stoppered with muslin gauze at 25°C and 80% r.h. The newly emerged flies were fed on rats or mice infected with *T. brucei*, EATRO 999, on day 6 or 7 after infection by the intraperitoneal route. This time of feeding (day 6 or 7) coincided with the first peak of parasitemia.

After feeding, flies were placed in a dark room at 20°C and 80% r.h. for 8 hours. After 8 hours the flies were moved into another room at 25°C and 80% r.h. At time intervals of 3, 4, 7, 9, 12, 13, 14, 22 and 96 hours after feeding 4 to 5 flies were dissected under sterile conditions and their intact gut placed in vessels containing spleen cells obtained from a bovine fetus and growing in RPMI 1640 medium with 25mM Hepes buffer, 20% fetal bovine serum 1% lactalbumin hydrolysate and antibiotics. The bovine cells, parasites and insect gut were grown at 37°C. Up to the 5th day of observation parasites in fly guts were seen strictly confined to the gut canal and by day 6 onwards parasites appeared in the medium outside the insect gut canal. Parasites only survived in the fly gut set at 12, 13 and 14 hours after feeding on infected animals. Such parasites are now maintained in continuous culture. When stained and enlarged 2,250 times, (Figure 1) the parasites appeared long and their kinetoplast (kt) was located between the posterior end and nucleus. In some cases the kinetoplast rested 2/3 way from nucleus to posterior end.

By the 8th day it was necessary to transfer some of the parasites to new vessels containing bovine cells. Thereafter parasites were subcultured every 48 to 72 hours and multiplication continued to occur in the presence or absence of tsetse gut. Later parasites could be incubated at 27°C with impunity. The population

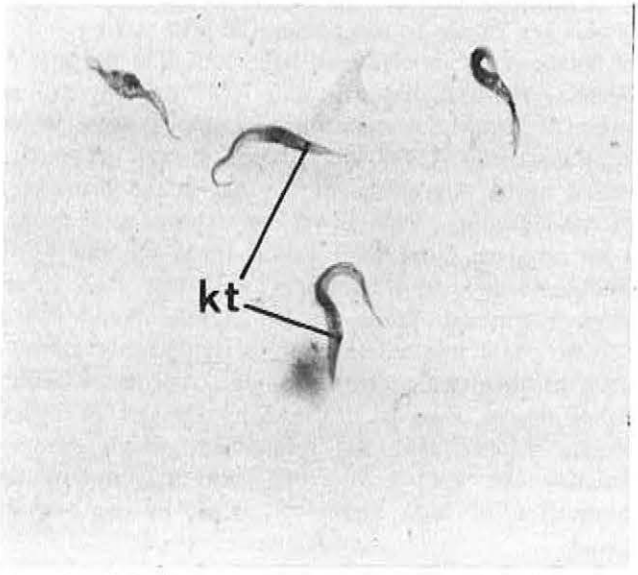

Figure 1. Mid-gut forms of *Trypanosoma brucei* on day 75 of propagation. The parasites are long and the kinetoplast (kt) is situated between the posterior end and the nucleus. Giemsa stained. (×2,250)

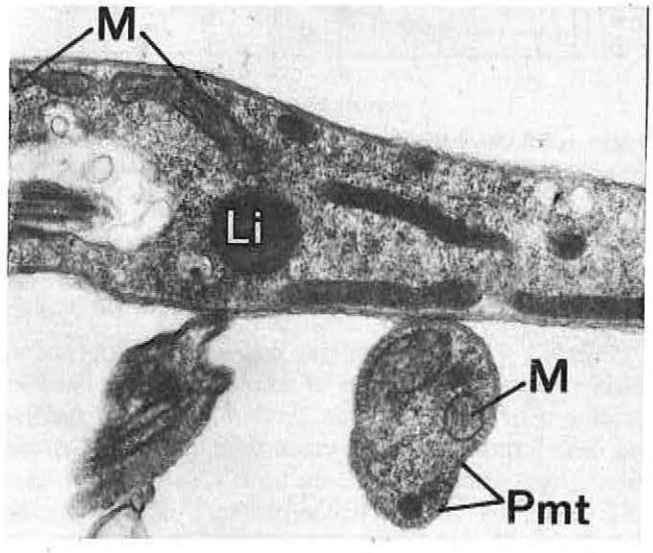

Figure 2. Electron micrograph of mid-gut culture forms of *Trypanosoma brucei* on day 24 of cultivation. There are well developed mitochondria (M) bordered by 2 membranes and containing tubular cristae. Lipid-like inclusion (Li) and pellicular microtubules (Pmt) are shown. (×30,000)

Table 1. Age of cultured mid-gut forms of *Trypanosoma brucei* inoculated into rats; prepatent periods and mortality rates after inoculation

Age of culture in days	Number of animals which became infected	Prepatent period in days	Number of animals which died of infection
4	2	10	1
10	3	8	0
18	3	9	0
20	1	6	0
27	2	7	0
31	3	5	0
34	3	6	0
40	2	11	1
46	2	12	0
49	3	5	0
61	3	14	0
75	0	—	0
88	0	—	0
124	0	—	0
149	0	—	0

Four animals were used in each group except on day 75, 88, 124, and 149 when 10, 6, 10, and 10 animals were used respectively

doubling time interval at 37°C was 4 hours and 12 hours at 27°C. Parasites transferred to vessels with no bovine cells died by the 10th day.

When cultured parasites at both temperatures of incubation were injected into rats as shown in Table 1, infection occured usually in 5 to 10 days. No differences in the infectivity was detected whether parasites were growing at 37°C or 27°C.

Electron microscopic studies of the cultured parasites (at 37°C) magnified 30,000 times (Figure 2) showed characteristics similar to those described by previous authors, ie. the presence of well developed mitochondria (M) lipid inclusions (Li), pellicular microtubules (Pmt).

Although these parasites lacked a surface coat and presented all the features of the non-infective forms described in the tsetse fly, it was possible to maintain infectivity up to at least 2 months of culturing. In some instances inoculated animals died. When flies were fed on the cultured parasites cyclical development occurred and infection was caused in rodents by the bite of such flies.

Attempts are being made to grow parasites from the salivary glands of tsetse flies.

The composition of the salivary gland secretion of the tsetse fly

N. Y. Patel

The use of electrophoretic and various chromatographic techniques for analysis of the salivary gland secretion of the tsetse fly was mentioned in the previous report (ICIPE Annual Report, 1976).

The secretion is a clear, transparent viscous fluid with a pH of between 7.0 and 7.5. About 500 probes of saliva secretion contained 14 µg of proteins, 5 µg of carbohydrates and 2µg of lipids.

Sixteen amino acids were identified by two way ascending paper chromatography in the saliva hydrolysate, of which aspartic acid, glutamic acid and glycine are major one. Cystein, cysteic acid, proline and tyrasine are found in trace amounts (Figure 1).

Analysis of sugars in hydrolyzed and unhydrolyzed (dissolved in 80% ethanol) saliva was performed by descending chromatography on Whatman No. 1 paper using butanol: acetic acid: water (4:1:1) solvent system. When the sugars were detected by an alkaline $AgNo_3$

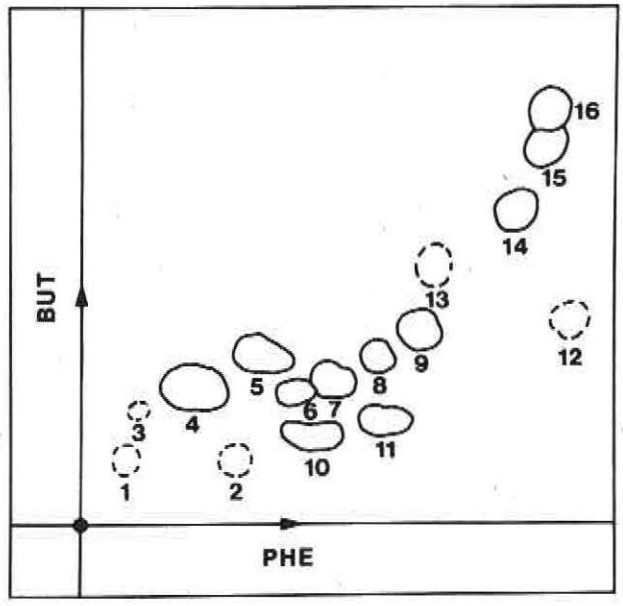

Figure 1. Two-way chromatographic separation of amino acids in an acid hydrolysate of tsetse saliva n-butanol/acetaic acid/H_2O-BUA (12: 3: 5 v/v/v); phenol/H_2O-PHE (4: 1 v/v) solvent systems 1. cysteic acid; 2. cystine; 3. asparagine; 4. aspartic acid; 5. glutamic acid; 6. serine; 7. glycine; 8. threonine; 9. alanine; 10. histidine; 11. lysine; 12. proline; 13. tyrosine; 14. valine; 15. phenylalanine; 16. isoleucine/leucine

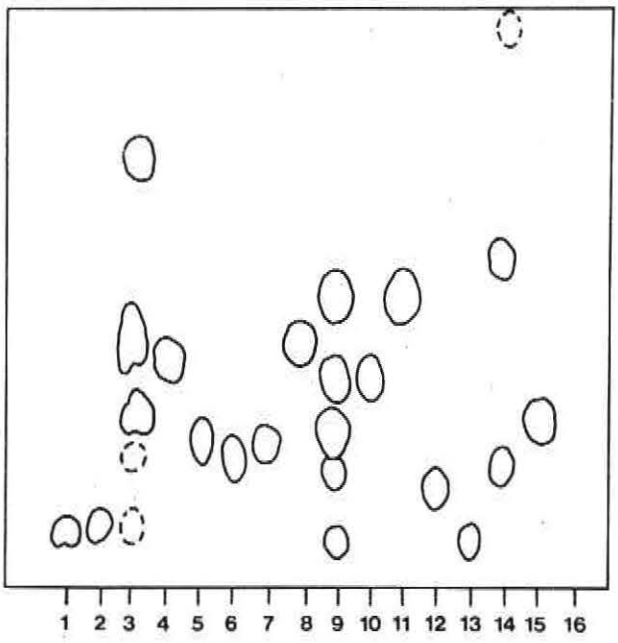

Figure 2. One dimensional paper chromatogram of hydrolysate of tsetse saliva run by descent for 18 hours. Solvent, n-butanol/ acetic acid/ H_2O (4: 1: 1 v/v)
1. inositol; 2. trehalose; 3. saliva; 4. arabinose; 5. glucosamine; 6. glactosamine; 7. galactose; 8. xylose; 9. mixture; 10. fructose; 11. ribose; 12. sucrose; 13. lactose; 14. glucoronic acid; 15. glucose

solution, five spots appeared (Figure 2). Four of these spots corresponded to inositol and/or trehalose, galactosamine, glucose and arabinose. The 5th spot appeared white with alkaline $AgNo_3$ solution. Identification of this spot requires further analysis.

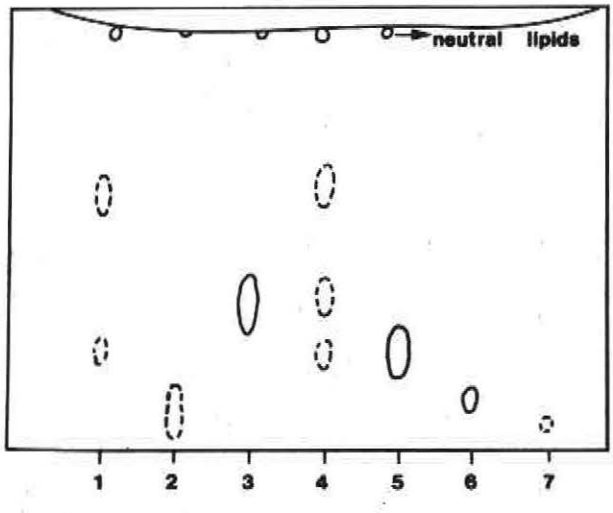

Figure 3. Diagrammatic representation of various phospholipids of tsetse saliva obtained on silica gel 'G' thin layer chromotograms. Solvent, Chloroform/methanol/acetic acid /H_2O (60: 30: 10: 3.5 v/v/v) 1-phosphatidyl ethanolamine; 2 phosphatidyl serine; 3. phosphatidyl inositol; 4. saliva; 5. lecithin; 6. phingomyelin; 7. lysolecithin

The three spots that appeared for phospholipid analysis by Thin Layer Chromatography (TLC) are identified as phosphatidyl ethanolamine, phosphatidyl inositol and lecithin (Figure 3).

None of enzymes, such as acid and alkaline phosphatase, general esterases, malic acid enzyme, malate and isocitrate dehydrogenases were detected when the gels were stained with the specific stains for these enzymes.

In order to analyse saliva droplets from one single fly. the gels were prepared in a 1mm diameter capillary tubing as described previously. Electrophoresis was conducted at 1.5 mA/gel for 10 minutes. The tubes were frozen for 2–3 minutes and the gels were ejected from the capillary with a microsyringe filled with water. About 50 probes from one fly were used for these analyses and the same results were obtained as that obtained by 2.5mm tubes.

Currently, similar analytical work on the composition of the saliva from tsetse salivary glands infected with trypanosomes has been started.

Tsetse trypanosomes interactions

L. H. Otieno

It has been observed that some strains of *trypanosoma brucei*, EATRO 1969, EATRO 2238, Lambwe 3 and Geneva SK 227 have different infective capacities for tsetse fly, *Glossina morsitans*. The invection rates range consistently from 0 (SK 227) to 11.6% (EATRO 1969). The most promising recent approaches to the intrinsic identification of protozoan parasites are the direct biochemical methods. Isoenzyme electrophoresis has proved particularly promising. The aim of the present work is to see whether there are any differences (isoenzyme patterns) that may be linked with strains that are more easily susceptible to tsetse fly or vice versa.

Polycrylamide gel electrophoretic studies of two strains of *T. brucei*, EATRO 2416 and 1969) have shown that these strains of trypanosomes have consistent differences in their isoenzyme patterns for two enzymes, malic enzymes (ME) and Glucose—6—Phosphate dehydrogenase (G6RD), out of six enzymes investigated. The two strains of trypanosomes differ sharply in their ability to be transmitted by *G. morsitans*. EATRO 1416 is highly virulent to laboratory animals (fatal within three days after inoculation) and does not develop to infective stage when ingested by *G. morsitans*. EATRO 1969 causes a chronic infection in laboratory animals and is easily transimitted cyclically. These investigations are being extented to various strains of *T. brucei* which have different susceptibility to tsetse flies in order to see if isoenzyme electrophoresis could be a useful tool in differentiating such strains.

Morphogenesis in *Trypanosoma brucei* after ingestion by *Glossina morsitans*

It has been known for some time that blood from *T. brucei* once ingested by *Glossina* either die or change into midgut form. These forms (midgut) are not infective to mammalian hosts until the insect phase of development is complete. Laboratory observations have shown that change from blood to midgut form is first detected about 8 hours following ingestion of an infected blood meal and reach a peak by about 24 hours. The blood form trypanosomes disappear completely from the midgut within 48 hours.

In vitro studies have revealed that incubating ingested blood (at a particular stage of digestion) from old *G. morsitans* with blood form *T. brucei*, results in a similar morphological change (transform into midgut form, in a few cases epimastigotes may also be observed). The transformation takes place within 10–15 minutes and no further change is observed by incubating for more than one hour.

In some cases, apart from the transformation there is a tendency for the blood form trypanosomes to stick together in a rosette form. This behaviour appears to be associated with degeneration and is stimulated with by-products of digestion, as it is seen only when the gut contents have turned yellowish green.

Attempts are being made (mainly through sephadex gel filtration) to isolate the active compound which initiates the morphological change and or rosette formation. This factor may well be used to interrupt *T. brucei* cyclical development within the tsetse.

FIELD WORK AT LAMBWE VALLEY

Some aspects of the biology of *Glossina pallidipes* were studied at Lambwe Valley, South Nyanza, Kenya. This study was a follow-up of a similar one made at Lambwe Valley in August 1975. The main objective of the study was to confirm some of the laboratory observations on the biology of *Trypanosoma brucei* in tsetse.

Langridge traps were used to trap the flies. All active flies collected were separated individually into plastic tubes and transferred to a laboratory (Homa Bay Secondary School). In the laboratory, flies were persuaded to salivate onto microscope slides and each fly that had salivated was numbered to correspond with the respective slide containing the saliva. The slides were fixed with methanol and stained with Giemsa's stain and later examined for the presence of trypanosomes. Those flies which could not salivate but were strong enough to feed were fed individually on immunosuppressed mice to allow easy infection.

The two groups of flies (those which had salivated and those which had been fed on mice) were then dissected and the proboscis, salivary glands and the entire gut examined for the presence of trypanosomes. Any tissue found infected was crushed in PBS pH 72 and inoculated into an immunosuppressed mouse. In this way it was possible to check each fly for the presence of trypanosomes either in the saliva or in the body tissues and also to see which fly was having infective trypanosomes.

Of the 543 flies examined, salivary secretions were collected from 341, of which 38 (11.1%) contained trypanosomes. Most of these belonged to the *T. vivax* group. *T. brucei* subgroup was identified in one case only. It was not, however, always possible to identify the species of trypanosomes because in some cases sphaeromastigotes were the only forms seen.

A comparison of saliva examination and fly dissection methods showed that the two methods were comparable. Dissection method showed that 10.9% of the flies were infected and saliva examination showed that 11.1% were infected. However, not every fly with infected saliva was also found on dissection to be infected. Of the 38 salivary infections only 32 were found to be infected on dissection.

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Studies on the brain neurosecretory cells, corpus allutum (CA) and corpus cardiacum (CC) of the tsetse fly, *Glossina morsitans morsitans*

J. Kawooya, R. W. Kunyiha and M. F. B. Chaudhury

Since the recent investigation in our laboratories clearly revealed that the various cyclical events of the tsetse reproduction process are under hormonal control, a research project was undertaken to study in detail the various neuro-endocrine glands of *Glossina morsitans morsitans* and to determine any cyclical activity of release and synthesis that may exist in these glands which may be related to various events in the reproduction cycle.

The description of the neuroendocrine system is based on the results obtained from paraldehyde fuchsin (PF), performic acid Victoria blue (PAVB) *in situ* preparations, as well as those from histological sections stained by alcian blue phloxine (ABP), azan and chrome haematoxylin phloxine (CHP).

The median neurosecretory cells (MNC) of the pars intercerebralis consist of 3 A-cell types and a single B-cell type. The lateral neurosecretory cells (LNC), situated in the pars intercerebralis lateralis, consist of a single B-cell type only. The neurosecretory material of all the A-cell types is stained positively with PAVB, PF, alcian blue and chrome haematoxylin stains, but the B-cells are stained negatively with these stains and are strongly phloxinophilic.

The axons from the MNC join medially to form a single median tract which runs for some distance within the protocerebral and deutocerebral regions. The axons of the lateral cells join to form the lateral tracts which, after running for a short distance join the median tract within the tritocerebral region. The single tract formed as a result of this fusion traverses short distances within the tritocerebrum and later divides outside the brain into two nerves, the nervi corporis cardiaci (NCC). These nerves initially take a course along the lateral oesophageal walls within the oesophageal canal but later come to lie intimately on the lateral walls of the aorta. The neurosecretory material of the A-cells is traceable in NCC and in the part of the aorta to which these nerves are attached. However, this material is not obvious in the CC which is known to be the neurohemal organ in other insects. This observation indicates that the aorta functions as a neurohemal organ in *Glossina*.

The MNC are observed to be actively synthesizing and secreting their products throughout the pregnancy cycle. This phenomenon is attributed to the events of egg maturation, ovulation, adult feeding and the uterine (milk) gland activity, all of which occur in succession and are considered to be under the influence of hormones from the MNC.

The corpus cardiacum is a single, bilobed, elongated gland which lies on the ventro-lateral aspect of the aorta. It is fused to the aorta by specialized syncitial tissue known as the cardiogeal tissue. Histological sections show that the CC consists of one to two layers of large cells which are arranged peripherally with a few others located within the centre of the gland. In the intercellular spaces of the CC and in the cytoplasm of the CC-cells, granules which stain positively with phloxine in the same way as the granular inclusions of the B-cells of the brain, are seen. It is probable that the neurosecretory material from the B-cells are stored in the CC.

The corpus allatum is a single ellipsoidal gland located on the dorsal aortic wall. The cells of the CA have prominent nuclei and nucleoli but poorly defined cell boundaries. A significant direct relationship is observed between the size of the CA and the weight of the fly. The CA is innervated by two nervi corporis allati (NCA), which seem to be the branches of the NCC. Smaller branches of the NCA ramify within the intercellular spaces throughout the gland. In CA, there is a peculiar large vesicle which is sometimes filled with neurosecretory material. The vesicle is considered to be a rudimentary space which has persisted as a result of the incomplete invagination of the gland during embryogenesis.

A detailed study of the CA volume during the first pregnancy cycle reveals that the CA increases in volume from about an average of 10×10^4 μ^3 at emergence to

about 21×10^4 μ^3 during ovulation and about 24×10^4 μ^3 during pregnancy with an early third instar larva. The volume decreases during the middle of pregnancy and at the time of larviposition. The CA volume of the virgin flies of the comparable age group does not show a significant difference when compared with the CA volume of the mated flies.

The presence of CA seems to be essential for the purpose of the development of ovary and the accessory gland of the mated tsetse flies (see following section). Although the virgin females do not show cyclic activity of the milk gland, the oogenesis proceeds normally in these flies. Because of the normal oogenetic activity, the CA of virgin females probably maintains a cyclic activity comparable to that observed in the mated flies.

Regulation of milk gland activity and larval development

M. F. B. Chaudhury, T. S. Dhadialla and F. Mukunza

The genus *Glossina* comprises one of the few groups of insects in which all of the nutrients necessary for the growth and development of progeny is transmitted to the larva while it is in the reproductive tract of the mother. The maturing larva is nourished in the modified bursa, the uterus, of the female by the secretion of the uterine gland, the so-called milk gland, a branched tubular structure opening into the uterus. Since the demand for vitellogenesis is little, the metabolism of the female fly is primarily directed towards the conversion of the blood meal into milk in order to nourish the maturing larva.

Dynamics of the milk gland activity in relation to blood meal size and larval development was studied and possible endocrine involvement in the control of milk gland activity was investigated. The changes in the transverse diameter of the distal tubules of the milk gland from the time of eclosion to the time of larviposition was observed in the normal females and was compared with those of the allatectomized females and allatectomized females receiving hormone therapy with C_{16}JH or a juvenile hormone analogue, ZR 515. Blood meal size and pupal weight was recorded from an additional series of females of comparable experimental conditions.

Results indicate that allatectomy at 12 hour post emergence does not effect ovarian development or mating but does effect the blood meal size, though not significantly (Table 1). Although the effect of allatectomy on the blood meal is not significant, the effect on the larval development is striking (Table 2). Allatectomy prolongs the interlarval period and reduces the pupal weight as recorded for the first four cycles. Since a series of normal females kept on restricted feeding (simulating blood meal size of allatectomized females) produce progeny of normal size at normal interlarval period, it is suggested that allatectomy, and not reduced blood meal, results in abnormalities in these cases.

Replacement therapy with the juvenile hormone, C_{16}JH and an analogue ZR 515, applied topically at a dosage of 0.2 µg per allatectomized female every alternate day, results in reproductive cycles of almost normal length and pupae of subnormal weight (Table 2).

Table 1. Blood meal size during the first four cycles of normal, allatectomized, sham operated, allatectomized and JHA treated female *Glossina morsitans morsitans*

Treatment	1st Cycle	2nd Cycle	3rd Cycle	4th Cycle
Normal ♀♀ (N=15)	250.6±26.1	201.7±15.8	210.2±9.3	208.5±22.7
—CA ♀♀ (N=20)	231.1±33.5	192.4±21.6	189.2±12.1	180.2±10.3
—CA + JHA (N=15)	241.5±20.2	202.0±16.9	197.3±21.2	205.1±13.7
Sham ♀♀ (N=12)	257.3±12.4	215:4±11.1	201.5±15.4	217.1±7.9

Table 2. Effect of allatectomy and subsequent juvenile hormone therapy on duration of cycle and pupal weight

Treatment	1st Cycle		2nd Cycle		3rd Cycle		4th Cycle	
	D (days)	PW (mg)	D (days)	PW (mg)	D (days)	PW (mg)	D (days)	PW (mg)
—CA	22.3	21.7	12.8	18.1	12.5	15.5	14.1	16.1
—CA+JHA	21.3	23.4	9.2	25.7	10.1	26.6	10.0	26.8
—CA+C_{16}JH	20.5	24.8	9.3	26.5	9.5	27.4	9.8	27.0
—CA+Solvent	22.6	22.5	12.9	19.0	13.1	17.2	13.5	17.1
Sham	18.2	28.5	9.1	29.2	9.3	30.1	9.0	28.2
Untreated	18.1	29.3	9.3	28.5	9.4	28.8	9.1	29.2

0.2 µg JH/JHA applied topically alternate days. N=20

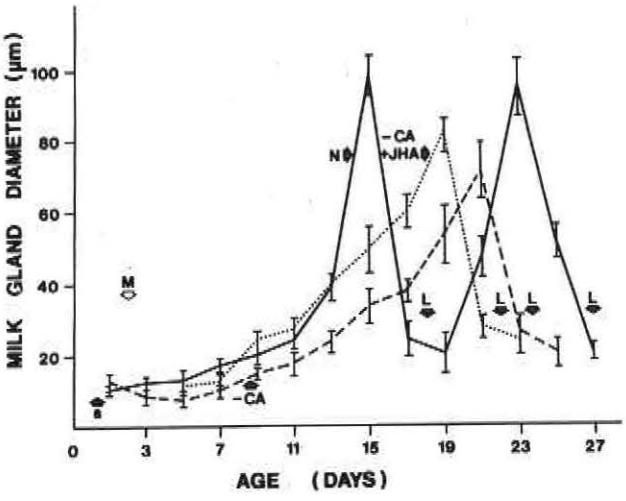

Figure 1. Diameter of distal tubules of the milk gland of normal, allatectomized and allatectomized but juvenile hormone analogue (ZR 515) treated female *Glossina morsitans morsitans* of various age groups

The milk gland undergoes dramatic cyclical changes which are correlated with the pregnancy cycle (Figure 1). These changes are clearly reflected in changes of the transverse diameter of the tubules which make up the entire gland. These changes are seen in both proximal and distal tubules but are more obvious in the latter ones.

The diameter of the distal tubules of the milk gland in 1 day-old normal flies is about 10 µm which increases gradually to about 20 µm by the time of the first ovulation. Following ovulation, the gland increases rapidly to a diameter of about 100 µm at about 3 days before the larviposition and then decreases abruptly to about 20 µm just before the larviposition (when the females are about 18 days old). During each of the subsequent cycles the gland increases to a maximum over the first 6 day period and decreases to a minimum just before each larviposition. The diameter of the milk gland tubules of the allatectomized females, on the other hand, increases only to a maximum of an average of 67 µm at about 4 days before the larviposition. The length of the first cycle is prolonged to 23–24 days and the second cycle to about 12 days. It is observed that the secretory vesicle and the lumen of the tubules of allatectomized females contain little secretion. Sham operated females show normal milk gland activity. Topical application of JHA (ZR 515) at a dosage of 0.2 µp per allatectomized female every alternate day results in tubules of larger diameter with an average maximum of 85 µm about 3 days before the larviposition.

From the findings described above, it appears that the allatectomy results in the failure of the development of secretion characteristic of the normal flies. Additionally, it appears that the cyclical changes of the volume of CA (preceeding section) in a pregnant fly, reflects the cyclical fluctuations in milk gland diameter. It is probable that allatectomy (thus lack of JH or a low titre thereof) affects the synthesis of milk leading to the production of diminutive offspring. Additionally, frequent hypertrophy of the fat body of the allatectomized flies suggests that the allatectomy may have an effect on the lipid metabolism in the fat body thereby affecting the milk synthesis.

Chemical analysis of secretions from the reproductive glands of male tsetse flies and of spermatophores

T. S. Dhadialla, M. F. B. Chaudhury and K. Endo

Some studies on the chemical analysis of the accessory reproductive gland (ARG) secretions from the male tsetse were reported in the first ICIPE Annual Report. As a continuation of that project, secretions from the ARGs and testes and the spermatophores have been analysed by chromatographic methods to investigate their amino acid compositions and the lipid and phospholipid contents of the ARG and testicular secretions. The protein pattern of spermatophores, collected from mated females, has also been determined by electrophoresis. Details of chromatographic and electrophoretic methods have been described by Mrs. N. Patel in the fourth ICIPE Annual Report.

Two dimensional (2-D) ascending paper chromatography of 80% methanolic extracts of secretions from the ARGs and testes of 8 day old unmated males did not reveal free amino acids. Spermatophores collected from the uteri of 2–3 day old mated females were washed and hydrolysed with 6N HCl for 24 hours at 110°C. Secretions from the ARGs and testes were similarly hydrolysed. The hydrolysates were washed with distilled water and then subjected to 2-D ascending paper chromatography. The major amino acids (indicated by staining intensity) present in the spermatophore hydrolysate were aspartic acid, glutamic acid, histidine, leucine/iso-leucine, lysine and serine. Trace amounts of cysteine, glycine, threonine, α-alanine, tyrosine, proline, valine, methionine and phenylalanine were also present. The amino acid composition of the ARG and testicular secretion hydrolysates resembled, except for the absence of methionine, phenylalanine and glutamic acid in the secretion of testes. Conversely, valine and leucine/iso-leucine were not detected in the hydrolysates of ARG secretions. Amino acids that were common both to the ARGs and testicular secretions were aspartic acid, histidine, lysine, serine, glycine and α-alanine. On comparing the bound amino acid composition of the secretions from ARGs and testes with that of the spermatophores, the amino acids cysteine, tyrosine, threonine and proline were detected only in the hydrolysates of the spermatophores. These additional amino acids may be products of biochemical reactions which lead to the alteration of proteins and hence polymerization of a spermatophore.

Twenty spermatophores collected from mated females were first briefly rinsed in 0.9% NaCl and then thoroughly washed in 40µl 0.9% NaCl. The washed spermatophores were homogenized in 40µl 0.9% NaCl, the homogenate centrifuged and the supernatent collected. The supernatent and the washings from the spermatophores were then used for electrophoresis (electrophoresis technique, conditions and staining methods were similar to those reported by Mrs. N. Patel in the fourth ICIPE Annual Report). The protein patterns of secretions from the ARGs and testes were also determined by electrophoresis for comparison with protein patterns obtained from the supernatents.

Supernatents from homogenized washed spermatophores separated into 10 stainable protein fractions of which 5 were major proteins (as indicated by the staining intensity). Thirteen protein bands were separated from the washings of spermatophores, while the secretions from ARGs and testes fractionated into 17 and 6 protein bands respectively. The protein patterns obtained from the supernatents of homogenized spermatophores, spermatophore washings, ARGs and testicular secretions were distinct and different from each other. Even though the proteins forming spermatophores are derived from ARG secretions, the different protein pattern of the homogenized spermatophores is probably due to the various chemical reactions which ultimately lead to the polymerization of the ARG secretions into a spermatophore. The washings of newly formed spermatophores would represent extracts containing proteins which are transferred as seminal fluid along with the spermatophore. The difference in protein pattern of the spermatophore washings from those of the ARG and testes secretions' protein composition may also be due to some chemical reactions resulting into different types of proteins. However, inspite of these differences in protein pattern, two of the protein bands from the spermatophore washings had similar electrophoretic mobilities to two protein bands from the ARG secretions. These two protein fractions from ARG secretions, if also identical in their amino acid compositions to the corresponding proteins from spermatophore washings, may represent the proteins that are transferred unchanged during the mating process.

Lipids and phospholipids from chloroform-methanol (2:1) extracts of secretions from the ARGs and testes were separated by thin layer chromatography and the various spots identified by separating a set of standards on the same plate as for the samples. Spots with Rf values similar to a monoglyceride (monopalmitin), cholesterol, triglyceride (tripalmitin) and a cholesterol ester (cholesterol palmitate) were identified from extracts of secretions from the ARGs. A single spot corresponding to the standard mono-palmitin was separated from an extract of the testes.

While trace amounts of phosphatidyl compounds of choline and serine were detected in the extracts of the testes, phosphatidylethanolamine, phosphatidylserine and phosphatidylcholine were present in extracts of the ARG secretions.

Investigations of a similar nature are in progress to identify the sugar composition of the secretions from the ARGs and testes and also of the spermatophores. Experiments to study the presence of the Krebs Cycle substrates in the ARG secretions and the relevant enzymes in the testes are being carried out. It is hoped that results of these investigations will give us a better insight as to the functions of the ARG secretions, other than that of spermatophore formation, for example, sperm motility (refer first ICIPE Annual Report).

Endocrine studies on male tsetse flies

It has been shown in a number of insects eg. *Schistocerca gregaria*, *Aedes aegypti* and *Periplanetta americana*, that secretory development of the accessory reproductive glands (ARGs) is under the control of corpus allatum (CA). Preliminary experiments have been carried out to investigate if such a control mechanism exists in case of the male tsetse fly. To determine if the CA hormone controls the secretory development of the ARGs after emergence, allatectomized males have been tested for their spermatophore forming and inseminating capability, since male tsetse flies having their ARGs surgically removed are unable to form spermatophores (third ICIPE Annual Report).

Newly emerged males were allatectomized within 10 minutes after emergence. These males were kept away from females in separate cages and given a blood meal everyday. On the 8th day, by which time normal males possess fully developed ARGs and are capable of transferring spermatophores to females, the allactectomized males were individually put together with single 2 day old virgin females in glass vials. Within half an hour of the completion of mating, the females were dissected to look for the presence of spermatophores in the uterus and sperm in the spermathecae.

Preliminary results indicate that allatectomy of newly emerged males did not effect the secretory development of the ARGs, since such males were able to inseminate up to 5 females each. None of these males, however, were tested for their fullest capacity to form spermatophores. The results of these preliminary experiments indicate that if the secretory development of the ARGs is under the control of CA, there might be enough CA hormone already present in the haemolymph at the time of adult emergence. Further experiments along this line of research are in progress.

POPULATION DIVERSITY

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Population diversity in the tsetse fly *Glossina pallidipes Aust*

J. van Etten

Several of the comparative studies of *Glossina pallidipes* populations in two selected areas, Nkruman and Mwalewa forest (Coast), have been continued (ICIPE Annual Report, 1976).

Trap catches

The comparative studies on the efficiency of two traps (Langridge Box Screen (=LBS) and Awning Screen Skirt (=ASS=Moloo trap) and the standing car have been finished. The main conclusions can be summarized as follows:

Within the Nkruman area the ASS trap catches significantly more flies than does the LBS, while the percentage of females in the total catch is significantly higher in the ASS.

Within the Mwalewa forest area there is no difference in the efficiency of the two traps, neither in numbers nor in the percentages of females.

The four traps used (2 LBS traps and 2 ASS traps) are on the average catching the same number of flies in both areas, but in Mwalewa forest the percentages of females in the total catch is significantly higher.

The standing car catches significantly more flies in Nkruman, while contrary to the trap, the percentages of females in the total catch do not differ in the two areas.

Based on the results of the trap and standing car catches we can conclude that the population size of the Nkruman area is fluctuating 3 to 4 times more than the population size in the Mwalewa forest.

Results from the trap catches are difficult to interprete because factors determining the trap catches are not yet known. This makes it difficult to understand whether the differences found in the trap catches in the two areas are due to external factors only, or are based on genetic differences in behaviour.

Diurnal activity

In the ICIPE Annual Report, 1976, it was reported that the diurnal activity patterns of flies from the two study areas are constant and, apparently, independent of temperature. The study on the diurnal activity patterns has recently been finished and analysis of the available data indicate that this conclusion has not been completely correct. The activity pattern of Nkruman is influenced very little by temperature, although the afternoon peak increases with increasing temperature to 36°C). However, the activity pattern of flies from Mwalewa forest is influenced much more by temperature. It was found that the midday depression only exists at midday temperatures of 30°C and higher. At midday temperatures below 30°C the midday depression disappears.

The results indicate that endogenous activity patterns are influenced by external factors such as temperature. Studies on spontaneous activity patterns under constant laboratory conditions and the factors which influence these patterns have been initiated and might lead to a better understanding of the field observations.

Nutritional studies

Females from the coast take more frequent bloodmeals than females from Nkruman do. They take, on the average, meals of the same size and this results in a

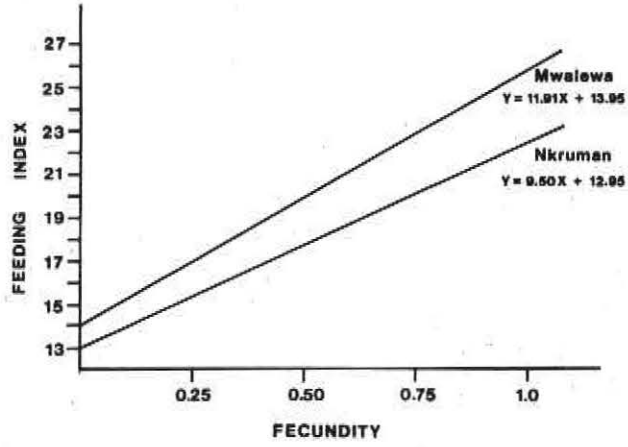

Figure 1. Correlations between fecundity and feeding index of females from laboratory colonies which originated from the two different populations

higher total blood intake by the coastal females. This was reported last year and it was indicated that the difference in feeding frequency has a genetic base.

The possibility that differences in blood intake result in differences in fecundity has been tested (Figure 1). The regression lines indicate the correlations between fecundity and feeding in the females of the two populations. The correlation is in both cases highly significant ($P>0.01$). At the same fecundity, the amount of blood taken is higher in the coastal females, confirming the original idea that differences in blood intake should result in a difference in the fat reserve in the females.

The studies on fat reserve also provide a good example of the differences in magnitude of the intra- and inter-populational diversity in the two populations under study (Table 1).

In Nkruman, observations were made on the fat reserves from flies from three different localities and the results were compared with those from the Coast. The results confirm that females from Nkruman have lower fat reserves than females from the Coast. However, the males from Nkruman show a greater variation in their fat reserves. The males from area C seem to have higher fat reserves than males from A and B, and these reserves are more comparable with the fat reserves in the coastal males. However, males from Nkruman originating from flies collected at area A, take the same amount of blood under laboratory conditions as males from the Coast. In addition, a preliminary experiment carried out with males which had emerged from field pupae from Nkruman, showed that the average fat reserve was 33.5% (n=16) after 20 days feeding under laboratory conditions. From these results it can be concluded that males from area C had optimal feeding during several hunger cycles, while males from A and B were less successful in obtaining their meals.

The same result is also shown in Table 2. The fat amount of each sample has been expressed as a percentage of the maximum fat amount obtained from each sex in both areas. Females from both areas and males from the Coast are equally successful in obtaining blood meals, but samples show that males from Nkruman are significantly less successful in obtaining meals. This makes it necessary to revise the conclusion drawn in the last Annual Report that traps catch different samples of males in the two areas, concluding now that males from Nkruman, for as yet unknown reasons, are less successful in obtaining their meals.

In summary it can be stated that in the past year, additional information on the existance of population diversity has been obtained and that, clearly, in one example the difference between intra- and inter-populational diversity could be indicated.

Table 1. Summary of the fat contents of samples of *Glossina pallidipes* collected with three different catching systems in Mwalewa (the Coastal area) and in three different localities in Nkruman (A, B and C, see text)

Catching system	Date		Mwalewa		Nkruman A		Nkruman B		Nkruman C	
			♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
Traps	July/August	1975	43.0	23.4	24.6	10.9				
	March	1976	—	24.4	27.7	14.5				
	May/June	1976	21.8	21.4	10.6					
	November	1976	—	—	—	—	31.2	23.4		
Car (Standing)	July/August	1975	43.5	23.9	14.4	10.3				
	November	1975	—	—	26.5	9.4				
	March	1976	53.2	33.3	28.5	14.7				
	May	1976	49.8	32.3	—	—				
	June	1976	55.5	33.2	27.7	10.8				
	July	1976	30.9	28.8	19.3	13.4				
	August	1976	43.4	39.7	39.1	24.5				
	September	1976	—	—	32.1	19.6				
	14–19 November	1976	—	—	—	—	35.9	24.9		
Car (Moving)	June	1976	—	—	27.3	17.3	—	—		
	October	1976	—	—	—	19.3	—	15.3	—	—
	5 November	1976	—	—	—	23.7	—	28.8		
	14–19 November	1976	—	—	—	16.7	—	—	37.1	34.9
	December	1976	—	26.7	—	—	—	—	—	—

Table 2. The average of all fat percentages obtained in all collected samples of both sexes from the two areas under study, expressed as a percentage of the maximum fat percentage found

	N	Average Fat-%-Age (F)	Maximum Fat-%-Age (M)	F. as %-Age of M.
♀♀ Nkruman	14	28.0±6.8	38.1	75.3±18.8
♂♂ Nkruman	19	18.1±7.1	34.9	51.8±20.3
♀♀ Mwalewa	8	43.8±9.1	55.5	79.0±16.4
♂♂ Mwalewa	10	28.8±5.7	39.7	72.4±14.5

CHEMISTRY AND BIOCHEMISTRY RESEARCH UNIT

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Chemical basis for termite resistance in wood

C. K. Wilkins

The wood from various trees is described in *Kenya Trees and Shrubs* as moderately termite resistant, very termite resistant, etc. A chemical examination of wood extracts to discover the causes of resistance or non-selection is underway. Trees indigenous to the Nairobi area were selected first for ease of collection. *Markhamia platycalyx* (Bignoniaceae) *Rawsonia lucida* (Flacourtiaceae) and *Bridelia micrantha* (Euphorbiaceae) were collected in the Karura forest with the assistance of Mr. S. P. Kibuwa and Mr. Kahurananga of the East African Herbarium.

The study of various experimental procedures for bioassays was carried out with the generous collaboration of O. Bruinsma. It was clear that at least two types of repellants exist; volatile and non-volatile repellant compounds. The presence of volatile repellant compounds is detected readily and often stimulates touching of the antennae to the source. Non-volatile repellant compounds seem to require the grasping of particles containing the compounds. (smell and taste?)

Volatile compounds are tested by placing workers (70–100) in a petri dish containing a piece of filter paper which covers the bottom. The paper is cut in half and part is treated with solvent while the second part is treated with a solution of the test substance. Five to 10 minutes standing in the dark is sufficient to show a significant difference in the population of workers on the two sides of the dish.

Solutions of non-volatile compounds are evaporated onto cellulose powder and the moist powder offered as building material in two piles on either side of the *Macrotermes* queen with two solvent treated cellulose powder piles. Counting individual instances of building behaviour by workers (100–200) over a period of 30–50 minutes showed that the workers could discriminate between treated and untreated cellulose while no difference was noted in the filter paper bioassay. Often treated cellulose bits are picked up and rejected immediately, behaviour which was not observed with untreated cellulose.

Extraction of *Markhamia platycalyx* and *Rawsonia lucida* sawdust gave small quantities of extractibles on concentration ($\eta 5.0\%$). Thin layer chromatography showed that the mixtures obtained were very complex with no predominant constituents. The whole extracts did show repellance in the building behaviour bioassay. Separation by column chromatography yielded active fractions but the small quantities of purified materials obtained was not promising. The ease of producing chromatographic fraction coupled with the difficulty of obtaining *Macrotermes* queens and workers also suggested that volatile termite repellants might be easier to study.

Since wood from members of the Cupressaceae is well known for its termite resistance, the African pencil cedar (*Juniperus procera*) was collected from the Rift Valley escarpment. Extraction of the heartwood yielded $\eta 10\%$ of a highly odoriferous gum. Column chromatography on silica gel and silica gel impregnated with silver nitrate yielded the major component α—cedrol as a crystalline solid. In the petri dish bioassay with *Odontotermes*, collected at Chiromo, cedrol was found to be highly active at concentrations comparable to its abundance in the wood. Further purification of various components and their bioassay is in progress.

Natural livestock tick acaricides and repellants

A. Maradufu

The control of livestock ticks by use of naturally occurring compounds is a research field which is just emerging. For all practical purposes, with the exception of pyrethrum, naturally occurring livestock tick acaricides and repellants are virtually unknown. Naturally occurring livestock tick acaricides and repellants when found will have obvious desirable advantages over synthetic compounds used for the same purposes. Because of their

occurrence in nature and hence the ease in their degredation they will not pose as environmental hazards or contaminants in milk or meat and other animal food by-products. Indeed, some of our preliminary chemical investigations do actually show that the repellants we have found in the plants are natural pheromones produced in other insects.

In this laboratory, investigations on the occurrence of naturally occurring livestock tick acaricides was begun by exploring the existance of such compounds in the gums secreted by *Commiphora* plants of Kenya. The gums are used by Somali people as insecticides and drug substances. The plants have now been identified as *Commiphora erythreae* and *Commiphora molmol* and all belong to the family *Burseraceae*. Both *C. erythreae* and *C. molmol* plants are found in the arid zones of the North Eastern Province of Kenya and in the neighbouring area extending into Somalia and Ethiopia. The gums are secreted on the outside of the stem trunk and are quite odourous. Freshly secreted gums are golden yellow in colour but darken to brown due to aerial oxidation. The native people skillfully collect the gums according to plant specie and sell them for insecticidal and medicinal purposes. The gums keep for a long time and are stored on the shelves of shop owners.

Our laboratory investigations show that gums of *C. erythreae* and *C. molmol* consistently contain the same compounds no matter how, who, when or where the gums are collected. Thus, although the gums from both plants appear the same except for the difference in odour, their collection is being carried out correctly.

Chemical analysis of the gums carried out together with bioassay tests reveal that the crude gums show dual properties as repellants and acaricides. Repellancy tests were carried out using larvae and adult ticks (males and females) of *Rhipicephalus appendiculatus* ticks. For the acaricide tests only larvae of *R. appendiculatus* ticks were used.

Results show that gums from both *C. erythreae* and *C. molmol* contain repellants and acaricides. The most active acaricide comes from *C. erythreae* gums. The repellants are of low molecular weight and less polar compounds than the acaricides, which have molecular weights ranging from 230 to 280. The acaricides are far more oxygenated than the repellants. Both types of compounds are sesquiterpenoids with a germacrane ring skeleton. The major structural variation among these compounds is brought about by differences in oxygenation patterns. Most of the compounds encountered so far are new compounds and a detailed chemical structural report will appear elsewhere.

Noteworthy in these investigations is the structural specificity of the repellants and acaricides. Structurally the two types of compounds are related. However, the physiological properties cannot be interchanged; that is, the acaricides show no repellancy properties and the latter are without any acaricidal activity.

It will be interesting in the future to study sensory organs involved in olfaction and hence to establish the mechanism employed by a tick to detect a repellant. Such electrophysiological studies should be important in the design of methods of application of tick repellants.

In our studies only *R. appendiculatus* ticks were used. The limitation here has been that other tick species are not available in large numbers at the moment. As soon as these are available our studies will be extended to involve other tick types.

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Mr. J. Owor (1973) Associate Scientific Officer

The unit was engaged in a large number of projects, inspite of its small size. The main lines of study are summarized below, but details of the investigations carried out in collaboration with other projects in the Centre will appear under appropriate sections in the report. Considerable work was accomplished with the following ICIPE scientists, namely:

Detailed SEM studies on the trifoliate organ of adults of the genus *Atherigona*

J. R. Clearwater (Sorghum Shootfly)

The characteristics of sensilla on the trifoliate process (external reproductive organs used in grasping females during mating) of several adult sorghum shootfly species were examined to facilitate their classification. As a result, an overall perspective of the genus as it occurs in Kenya was obtained and female specimens in this difficult group could be correctly placed taxonomically for the first time.

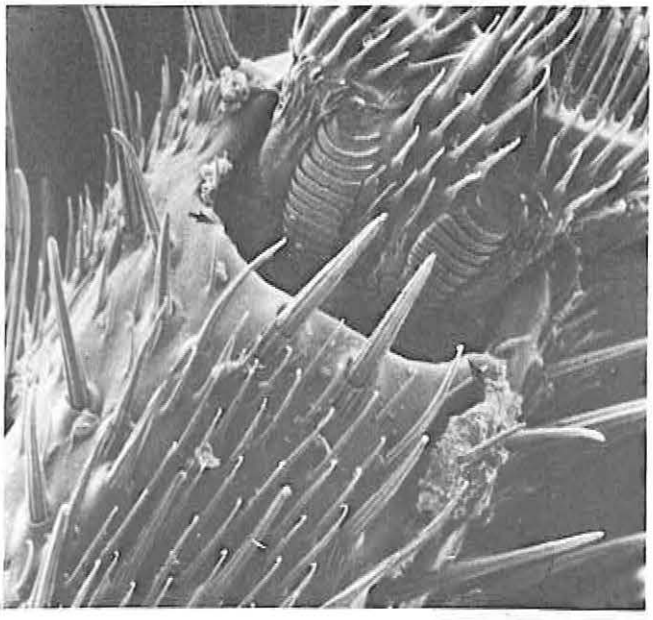

Figure 1. The last tarsal segment of *Atherigona soccata* showing the arrangement of the tarsal sensilla including two pairs of plate organs (arrows) (×3,000)

The morphology of the larval head capsule and the sensory structures on the adult ovipositor and tarsi in *Atherigona soccata* Rond.

K. Ogwaro (Sorghum Shootfly)

The arrangement and types of sensory receptors have been identified in the larvae and adult of *A. soccata*. The background ultrastructural information now obtained forms a foundation for further behavioural and electrophysiological studies. The results are shown in the micrograph (Figures 1, 2 and 3).

Morphology of pheromone-secreting gland in the armyworm

B. Otindo (Armyworm)

The external morphology of the female abdominal tip has been studied closely and related to the function of pheromone production (Figures 4 and 5).

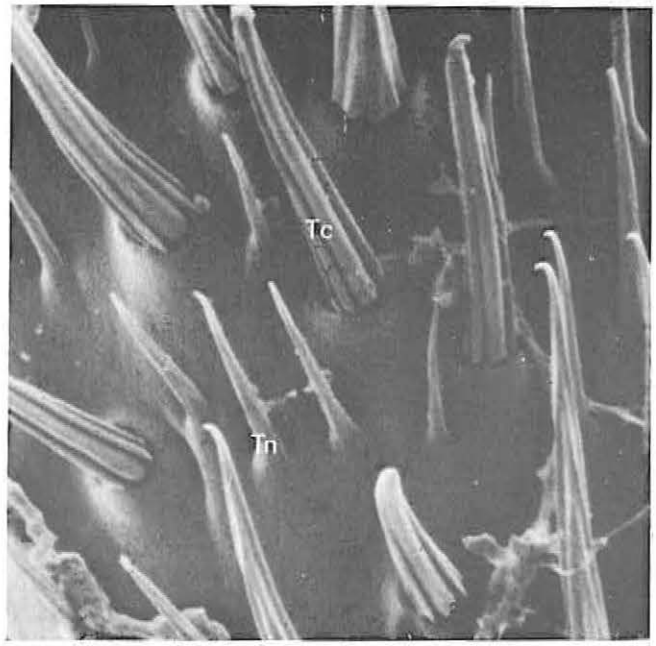

Figure 2. The thick-walled (Tc) and thin-walled (Tn) sensilla on the last tarsal segment of *Atherigona soccata* (×9,000)

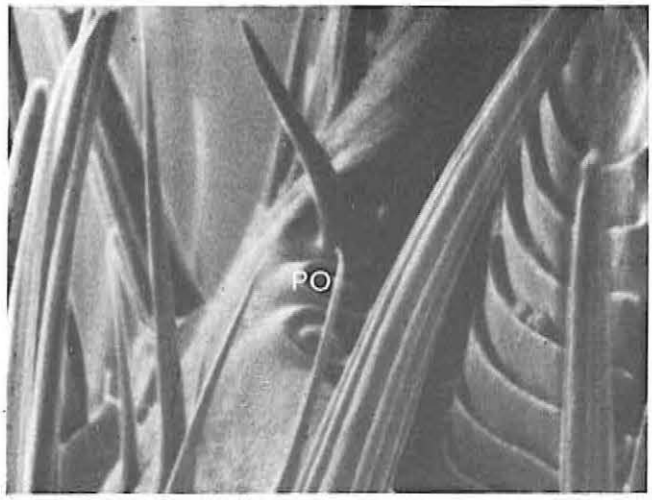

Figure 3. One of the two pairs of the plate organs (PO) on the anterior margin of the last tarsal segment of *Atherigona soccata* (×15,000)

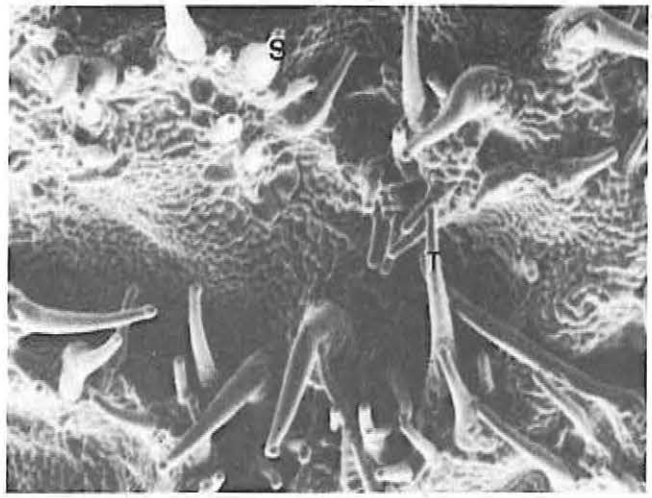

Figure 4. A portion of the pheromone producing gland of *Spodoptera exempta* showing two types of cuticular eversions (S=short spikes, T=tapering spikes) used for pheromone release. (×15,000)

Tsetse uterus

Mrs. J. Kongoro (Salivary Gland Physiology)

Research on the tsetse uterus was continued.

Pathogens of tsetse salivary glands

T. Jaensen (Tsetse Reproduction)

Based on TEM and cytochemical studies of enlarged tsetse salivary glands, DNA-containing and rod-like virus particles were identified in the cytoplasm of the extra-epithelial cells and lumen of *G. pallidipes* (Figure 6 overleaf). This is an important problem which merits further studies in tsetse reproductive behaviour.

Comparative study of the corpus allatum of different *Macrotermes* castes

M. Okot-Kotber (Termite)

The presence of rosettes, probably glycogen, was observed in the corpus allatum nucleus and cytoplasm of the 'soldier precursor' (Figures 7, 8a and 8b overleaf). These rosettes disappeared with subsequent larval development.

Activity pattern of *Glossina morsitans* corpus allatum during the 2nd pregnancy cycle

J. Kawooya (Reproductive Physiology)

An apparent alternation between 'lamellate' and 'lysosome-like' granules was observed (Figure 13 p. 65). The significance of these bodies is being investigated.

Sensilla on tsetse proboscis

E. Kokwaro (Histology and Fine Structure)

Four types of sensilla were identified and described on the haustellum of *G. morsitans* (Figures 9, 10, and 11 pp. 64-5). These may be significant in heat perception, and is related to the research project of Dr. Chaudhury.

Morphology of tsetse salivary glands

E. Kokwaro (Histology and Fine Structure)

SEM studies showed that the musculated distal portion of tsetse salivary glands, whose movement is controlled by musculature, is innervated by fine nerves (Figure 12 pp. 64-5) which originate from the thoracic ganglion. Further studies will be continued to determine the role of this nervous association during tsetse salivation.

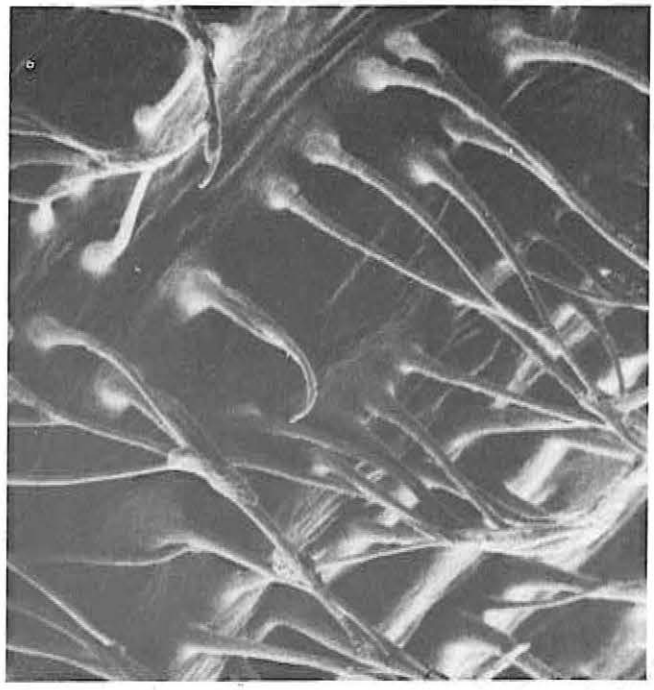

Figure 5. Cuticular eversions of the non-pheromone producing membranous region adjacent to the pheromone producing gland of *Spodoptera exempta* (×9,000)

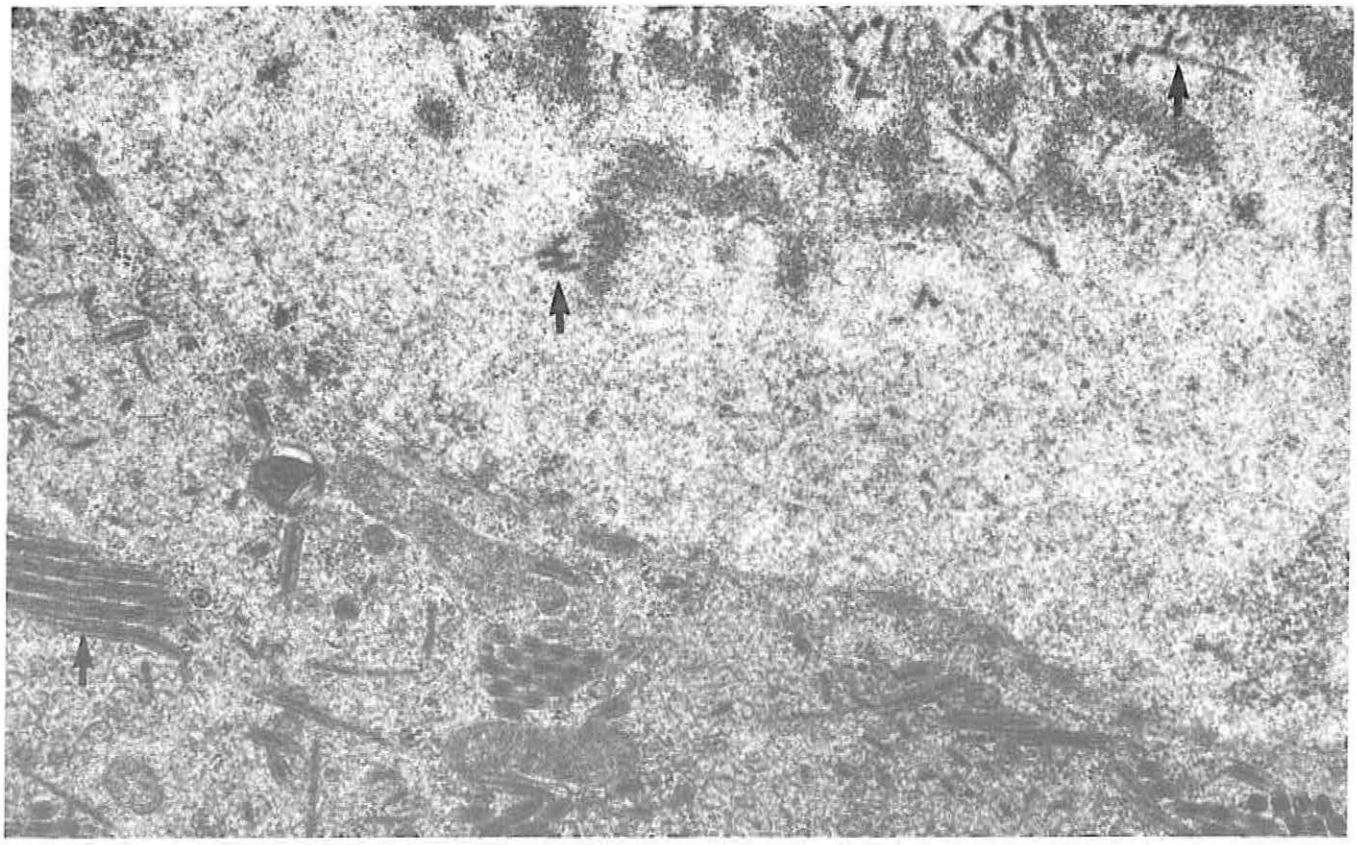

Figure 6

Figure 6. Virus-like particles (arrows) in the nucleoplasm, nucleolus and clytoplasm of the extra-epithelial cells of the salivary gland of *Glossina morsitans* ($\times$30,000)

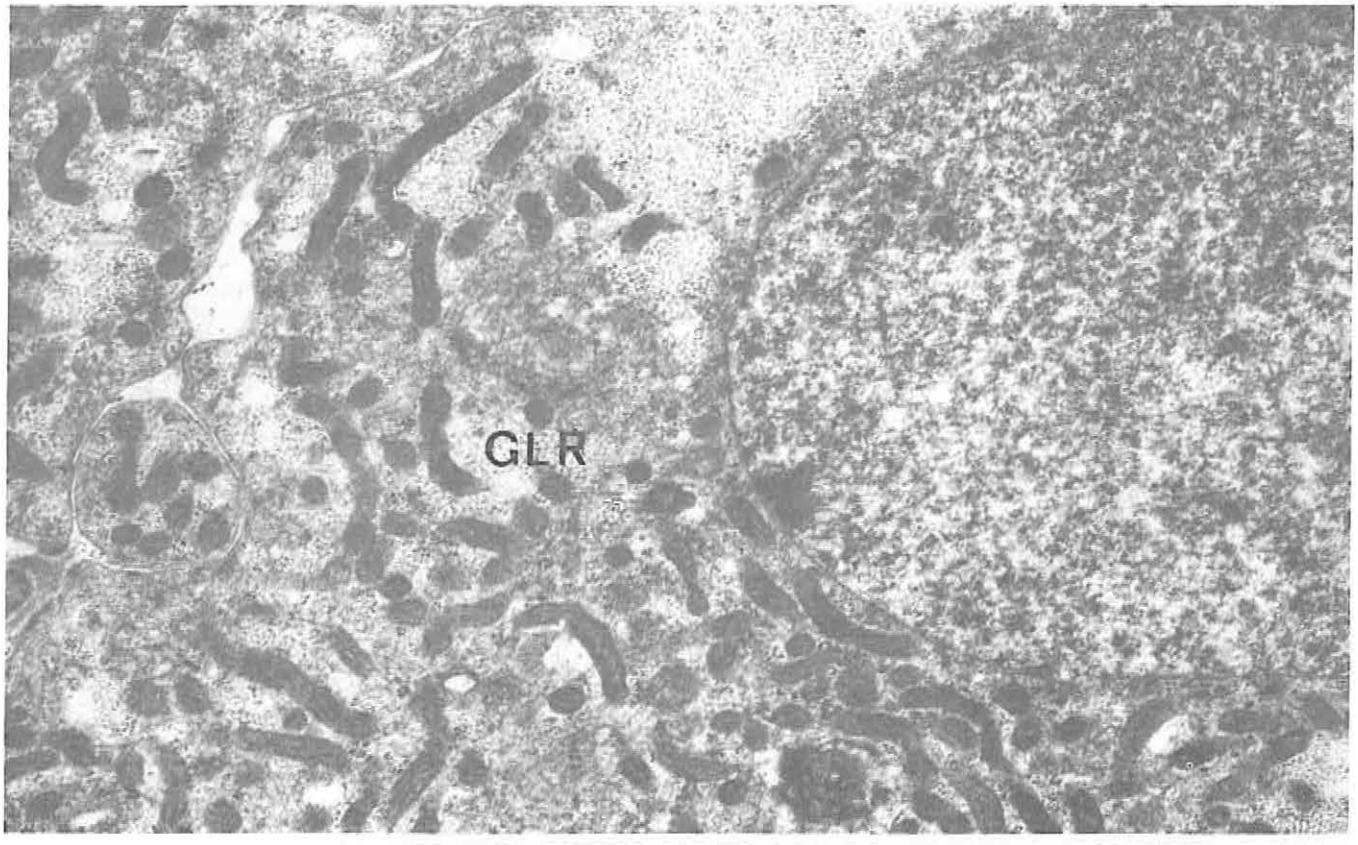

Figure 7

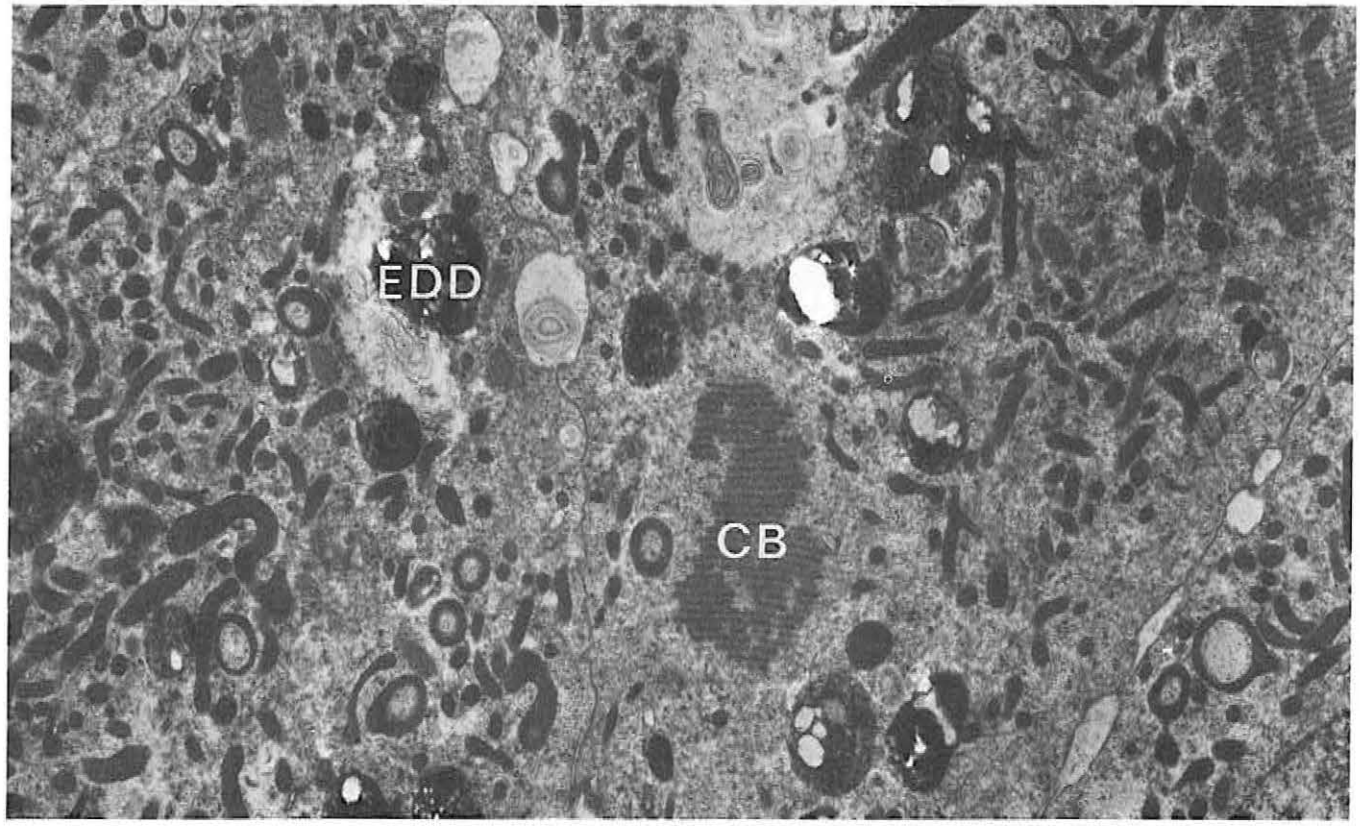

Figure 8a

Figures 7, (×31,000) 8a, (×11,000) and 8b, (×16,000) are micrographs of corpora allata "presoldier precursor", *Macrotermes* king and queen. The presence of "glycogen-like rosettes" (GLR) was observed in the "soldier precursor" (Figure 7) but not in the soldier
Figures 8a and 8b show rod-shaped bodies (RSB), crystalline bodies (CB) and electron dense droplets (EDD) with transculent bodies, which were observed in the CA of the adult *Macrotermes* queen, but were not present in the CA of male and female allates of 14 days and under

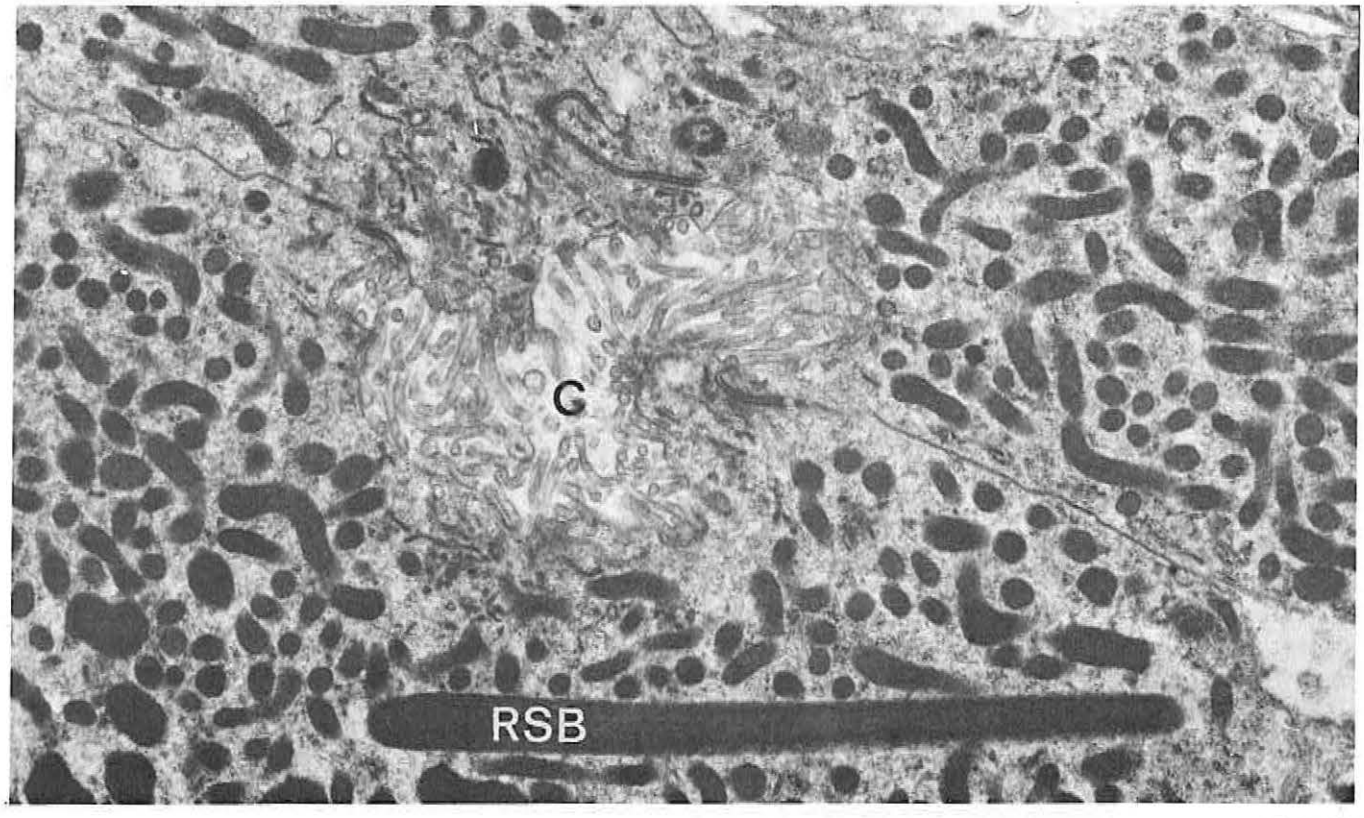

Figure 8b

Absorption in the gut system of *Glossina morsitans*

J. Owor (Histology and Fine Structure)

Flies fed with electron dense markers (colloidal carbon and ferritin) were examined to ascertain the presence of these markers in the different regions of the gut. Research on this is being continued.

Collaborative work was also extended to numerous other researchers outside the Centre. For instance, electron microscope studies with Dr. Walter Kaiser (Plant Quarantine Station, AFRO) on negatively-stained preparations from sugar cane, cowpea and cassava revealed that bacteria (Figure 14) are the causal agents of the ratoon stunting disease (RSD) in these plants. Ultrastructurally, it has been shown that several sensory cell units occur on the Haller's organ and palps of *Ornithodoros moubata.* (Dr. G. Karuhize, University of Nairobi). Other collaborative studies on the male accessory reproductive glands of the cotton stainer *Dysdercus fasciatus*, revealed changes in the cytoplasm with age (Mrs. L. Awiti, University of Nairobi). Ultra-structural studies on the dik-dik, camel and giraffe lungs and diaphragm were undertaken in collaboration with Professor E. R. Weibel and Dr. P. Gehr (Universities of Berne and Nairobi). In addition, training in basic EM techniques was offered to other members of staff acquainting themselves with the various instruments. Dr. A. Odebiyi (Research Associate from University of Ibadan) was trained in EM techniques and operation of the Jeol vacuum coating unit and the Jeol JSM—15 scanning EM.

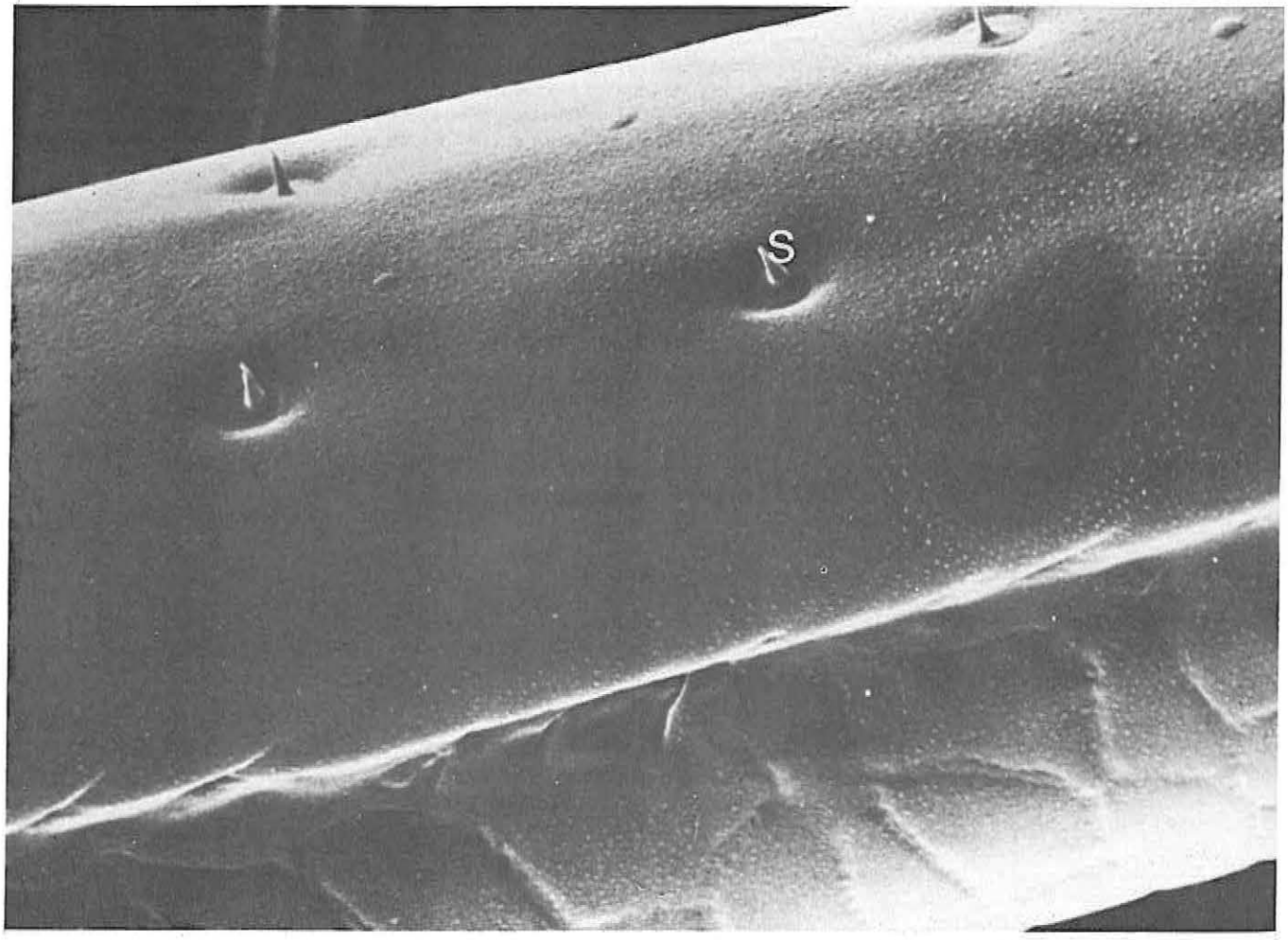

Figure 9. The ventral surface of the haustellum of *Glossina morsitans* showing two rows of sensilla (S) (×3,750)

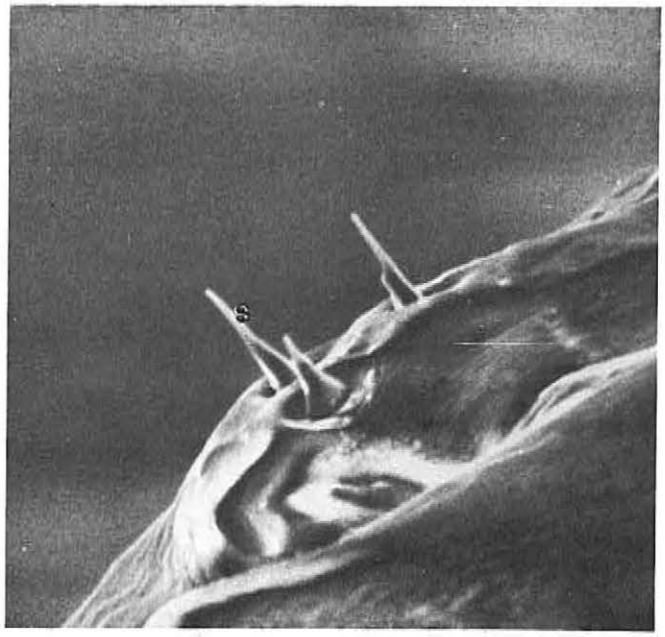

Figure 10. The junction of the haustellum with the labella of *Glossina morsitans* showing three sensilla (S) in a group (×18,750)

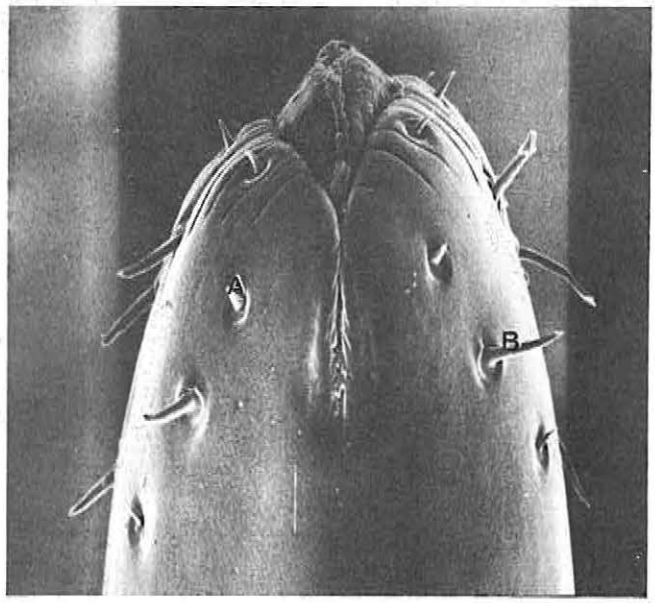

Figure 11. The labella of *Glossina morsitans* showing two types of sensilla (A, B) (×3,750)

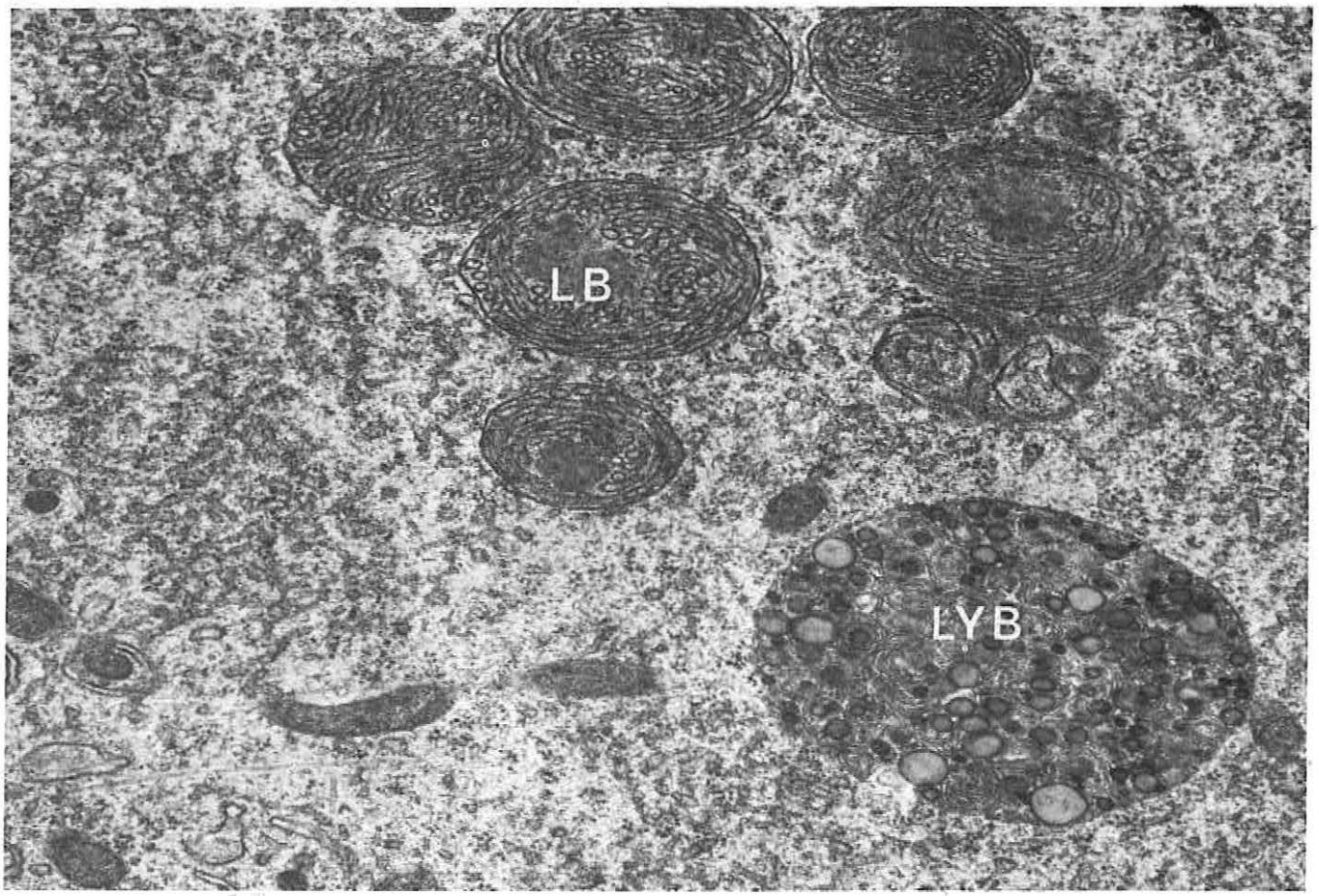

Figure 13. These two bodies—"lamellate body" (LB) and "lytic body" (LYB)—seem to alternate in abundance at any phase of the 2nd pregnancy cycle of *Glossina morsitans* (×31,000)

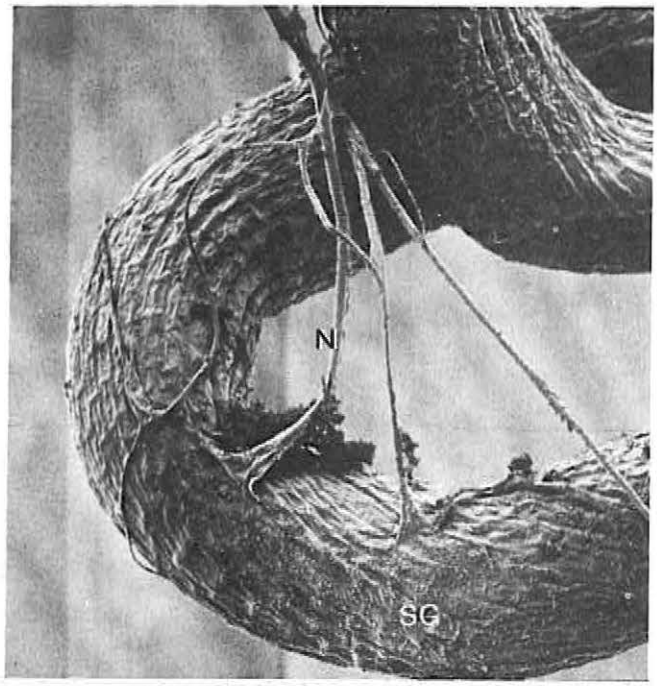

Figure 12. Nerve (N) supply to the tsetse fly salivary gland (SG) (×1,800)

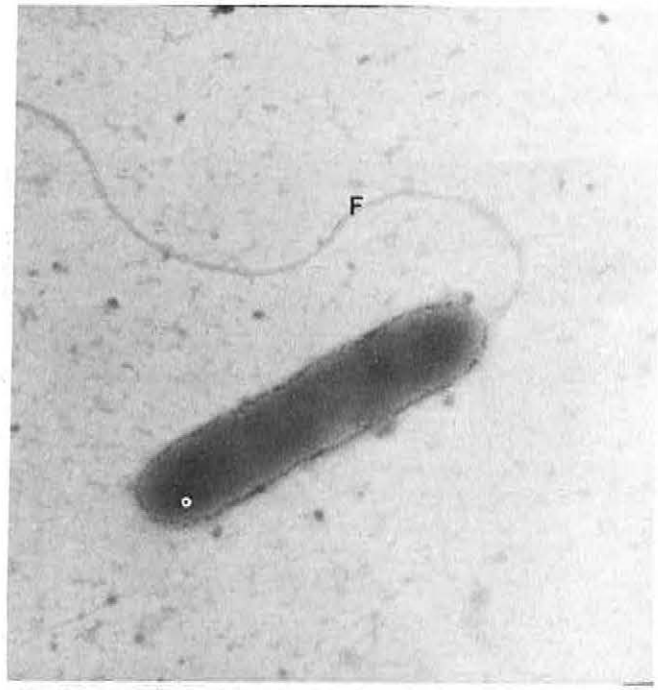

Figure 14. Electron micrograph of a single flagellated bacterium (F=flagellum). A vascular extract of RSD-affected cowpea was negatively stained with 1% sodium phosphotungstate (×39,000)

SENSORY PHYSIOLOGY RESEARCH UNIT

Visiting Directors of Research
Professor A. R. Møller (1973) Tsetse Acoustics
Professor T. R. Odhiambo (1970) Tsetse Acoustics
Professor D. Schneider (1970) Electrophysiology

Research Staff
Dr. J. V. Clark (1976) Postdoctoral Research Fellow
Dr. J. MacFarlane (1977) Research Scientist
Mr. H. M. Kahoro (1975) Technician
Dr. R. A. Steinbrecht (1975) Research Scientist
Mr. R. K. Saini (1976) Associate Scientific Officer

Collaborators
Dr. M. F. B. Chaudhury, Research Scientist
Dr. T. Fukushi, Visiting Research Associate—sponsored by the Japanese Society for the Promotion of Science

Styloconic sensilla

J. V. Clark

Work already performed at ICIPE (ICIPE Annual Reports 1974, 1975) has thrown a good deal of light on the receptor sensitivity of the African armyworm *Spodoptera exempta*, and has also revealed the sensitivities, in part, of the styloconic sensilla, which play an important role in the food plant selection behaviour of the caterpillar. These sensilla are located on the maxillary galea, both the lateral and medial sensilla being innervated by four chemoreceptor neurons and one mechanoreceptor neuron. The adequate stimuli for the four chemoreceptor neurons in the lateral sensilla have been described (two salt cells, one sugar cell and one adenosine sensitive cell), while only three have been accounted for in the medial sensilla (one sugar cell, two salt cells). No work has been done on the possible role of the antennae in the feeding activity of *S.exempta*, nor has any work been done on the role played by the sensilla located on either side of the spinneret, the labial palpi, neither in this insect nor in other species.

With the new electrophysiological unit set up in the sensory physiology research unit, work has been started on these topics, and furthermore, a bioassay routine has been arrived at for studying feeding activity of the caterpillars.

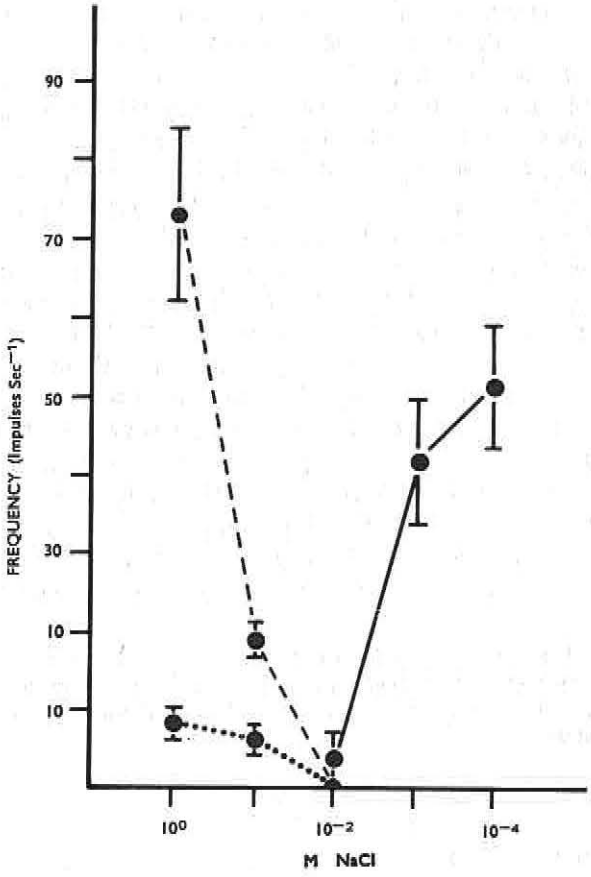

Figure 1. Receptor response of medial sensillum styloconicum to various concentrations of NaCl, represented as frequency of firing (ordinate) of the receptors, derived from the number of spikes within the first 500 msec after a 20 msec delay. The two salt receptors are below threshold at 10^{-2}M NaCl, whilst the water receptor increases in firing frequency with decreasing salt concentrations. Vertical bars represent standard errors

Styloconic sensilla

Electrophysiological recording from the medial styloconic sensilla of sixth instar larvae (using the tip recording technique) has revealed, in addition to the two salt receptors and the sugar receptor previously reported (Ma 1976), the presence of a water receptor, becoming active at concentrations below 10^{-1}M sodium chloride. Figure 1 shows graphically the response of this receptor, together with the already known salt receptors. It has not yet been possible to follow the activity of this receptor down to its presumed response to pure water, due to the attenuation of spike amplitude caused by the lowered conductivity of the stimulating solution at low salt concentrations.

Labial palps

These palpi are located one on either side of the spinneret, on the distal portion of the labium. Each palp bears

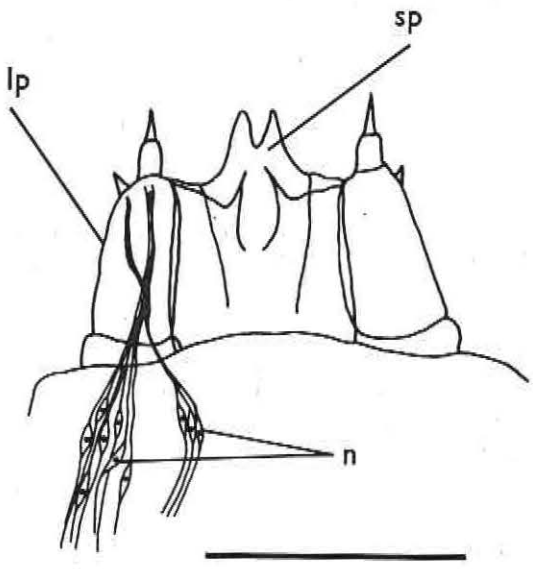

Figure 2. Camera lucida drawing of the paired labial palps situated on the basilar sclerite of the prementum, on either side of the spinneret (ventral view). Neurons innervating the sensilla, as revealed by methylene blue staining, are shown for the right labial palp (sixth instar larva)
Bar represents 100 μm
n=neurons
lp=labial palp
sp=spinneret

a sensillum terminally and a smaller sensillum laterally. Methylene blue staining of mouthparts of sixth instar larvae has shown that these palps are innervated, the lateral sensillum being associated with at least three, and the terminal sensillum with at least seven neurons. It has not yet been possible to determine if these neurons send dendrites right to the tip of the sensilla, nor whether the sensilla are open at their tip. However, it appears from their position and external morphology that these palpi may function as chemoreceptors, so for this reason attempts have been made to determine their sensitivity.

No responses have been found to crude extracts of various graminaceous plants, nor have any responses been recorded to a variety of sugars tested on the palpi. No response has been recorded to homogenized silk glands dissected out from pre-pupae. Occasionally small spikes can be recorded in response to salt, and in one case a definite response was obtained in response to a saturated solution of salicin. The failure to find consistent receptor activity may be because the adequate stimuli have not yet been found, or that these sensilla do not function in sixth instar larvae (cf. the styloconic sensilla have been found to cease responding to sugar and salt during the non-feeding stage preceding the pre-pupal stage), or indeed that these sensilla are not in fact chemoreceptors. It is intended to carry out further work on these receptors to find out what their role is in the activity of the caterpillar.

Bioassay

Some time has been spent in arriving at a suitable bioassay for studying the feeding response of sixth instar larvae. The basis of the bioassay is the agar/cellulose mixture of Hsiao and Fraenkel (1968), and after considering a number of possible indices of feeding activity (weight change of larvae, loss of weight of substrate, number of faecal pellets, weight of faecal pellets) it was decided that dry faecal pellet weight was accurate and the least time consuming of the possible indices. Sixth instar caterpillars are individually starved (with access to water) to empty their guts of plant material, and are then placed, one per dish, in 6cm diameter petri dishes together with two discs (1.4× 0.3cm) of agar/cellulose diet with the test material incorporated. A moist filter paper in the top of the petri dish reduces evaporation of the substrate by maintaining a high relative humidity. The caterpillars are placed in an oven at 30°C, and after feeding for 24 hours the faecal pellet production is measured by collecting and weighing the dried faecal pellets.

Using this bioassay the feeding response of sixth instar larvae has been compared with the responses obtained from the styloconic sensilla, using sucrose as the phagostimulant. Significant feeding responses to sucrose have been found at 10^{-2}M sucrose with the bioassay, putting the threshold of response between 10^{-2} and 10^{-3}M sucrose. This is similar to the response threshold to sucrose of the medial styloconic sensilla, recorded electrophysiologically, while significant responses to sucrose have been recorded from the lateral sensilla to sucrose concentrations as low as 10^{-5}M.

This bioassay has also been used to determine if plant odours have any effect on the feeding activity of sixth instar larvae. Larvae were presented with 0.1M sucrose in an agar/cellulose substrate, with and without the presence of leaf odours. The bioassay was run for 24 hours, and the crushed leaves (the source of the plant odours) which were placed within the bioassay dish but separated from the caterpillar by a nylon mesh, were changed twice during this period as they became less odorous with time. Two types of leaves were used, those of maize and those of castor, a plant whose

Table 1. Faecal pellet production of sixth instar larvae feeding on 0.1M sucrose in agar/cellulose discs with and without the presence of plant odours (crushed leaves). No significant difference between the means

	No. of larvae	Mean faecal pellet production (mg)	Standard error
Controls	17	6.8	±1.1
Crushed Maize Leaf	14	5.4	±1.1
Crushed Castor Leaf	17	5.9	±1.1

leaves are distasteful to *S.exempta*. The results of this assay, presented in Table 1, showed that, at least under the conditions of the experiment, the presence of crushed leaf had no significant effect on the faecal pellet production of the sixth instar larvae, suggesting that olfactory receptors did not affect the ongoing feeding activity.

References

Hsiao, T. H. and Fraenkel, G. (1968). Isolation of phagostimulative substances from the host plant of the Colorado beetle. Ann. Ent. S.A. **61**, (2)476–484

Ma, W. C. (1977). Alteration of chemoreceptor function in armyworm larvae (*S. exempta*) by a plant-derived sesquiter pinoid and by sulfhydryl reagents. Physiol. Ent. **2**, (3)199–208

Depression response of the haustellum by thermal stimulus in the tsetse fly, *Glossina morsitans morsitans*

T. Fukushi and M. F. B. Chaudhury

It is well known that some species of flies extend their proboscis in response to tarsal and labellar stimulation with sugars. In tsetse flies, probing involves two separable movements of the mouthparts: the depression of the haustellum and the extension of the rostrum. While the former one is a conspicuous movement, the latter is a small repetitive one. This paper describes the results of the experiments on the depression response of the haustellum by heat, which attempted to determine the region perceptive to heat, the threshold temperature and effect of extirpation of nerves on heat perception of the tsetse fly, *Glossina morsitans morsitans*.

As the heat source, a soldering iron tip (1.4mm diameter) connected to a variable transformer, or a fine copper wire tip (1.7mm diameter) whose temperature was regulated by a water bath, was used. To subject the fly to a certain temperature, the tip of the heat source was gradually brought close to the ventro-frontal part of the head of the fly which was securely fastened to a clay bed in the supine position. The tip of the heat source was kept about 0.5mm away from the body surface of the fly. The depression response of the fly was then recorded with various treatments and experimental conditions.

Nearly all flies tested responded to the heat source by depressing the haustellum. Heat stimulus for the response seems to be mainly perceived by the haustellum, ventral part of the rostrum and ventro-lateral part of the head. Within the haustellum, the labium was responsible for heat perception. Removal of the antennae, the major receptor site of heat, had little influence on the depression response of the haustellum to heat.

The threshold temperature to induce the response by contact of the heat source to the haustellum was 51–52°C. Severence of both ventral nerve cord and recurrent nerve caused an increased sensitivity to heat. It is probable that these nerves convey inhibitory signals to the central nervous system. Without these inhibitory signals flies may depress their haustellum in response even to the laboratory temperature (24–28°C).

INSECT AND ANIMAL BREEDING UNIT

Controller for Insectary Services
Dr. A. Basu (1976)

Technicians
Mr. J. Atema (1975)
Mr. E. Awuoche (1973)
Mr. H. Banda (1972)
Mr. A. Ikhunyalo (1971)
Mr. J. Kagoiya (1973)
Mr. J. Ongudha (1973)
Mr. J. Wanyonje (1970)

Report on activities in the unit

A. Basu and J. Wanyonje

Breeding of tsetse flies

Glossina morsitans

The breeding of *Glossina morsitans* was satisfactory during most of the year. Emergence of adults was normal in the first seven months, with a fall during the months of August, September and November. However, it again became normal from December (Table 1). Production of pupae was also very high during the first six months, with an average daily production of 309.17. But in the next six months production fell to 251.32 pupae per day. Pupal production increased again to 311.45 per day in the month of December (Table 2). There was also a considerable increase in the demands of flies and pupae during the year.

Glossina austeni

This colony was maintained mainly on pupae received from Bristol, the U.K. and France. It was found that the flies emerging from these pupae did not do well beyond F_2 generations. Due to lack of demand for this species. and also due to a shortage of space it has been decided to discontinue the breeding of this species in future.

Glossina pallidipes

Work is still proceeding towards the establishment of a satisfactory method for mass breeding of these flies in laboratory conditions.

Breeding of stemborers

Chilo zonellus

Due to a moderate demand for this insect, a small colony was maintained. The culture was maintained on an average of 500 larvae per month with monthly variations of 100 to 700. The death rate never exceeded 10%. The colony was maintained on an artificial diet with some modifications suggested by the previous scientist working on the project. A number of experiments performed by the previous scientist are still being continued in the insectary.

Besides this, at the request of the National Agricultural Research Centre, Kitale, the insectary tried to rear some larvae of *Busseola fusca* on the artificial diet for *Chilo*. A number of larvae were brought from Kitale in September and again in November. It was possible to rear them to adults, but it was not possible to mate the adults for laying fertile eggs.

Breeding of armyworms

Spodoptera exempta

This species comprises the largest colony of all armyworms. Two cultures, one fed on maize leaves and the other on an artificial diet were maintained. This colony did well almost continuously, except that some females failed to lay fertile eggs. These difficulties were rectified however, by the usual trial and error method. With the decrease in demand, some newly emerged moths had to be killed during December.

Spodoptera littoralis

A small culture of this species was maintained mainly on castor leaves and was used predominantly by people from outside.

Spodoptera triturata

A small culture of this species was maintained throughout the year although there was no demand for them.

Spodoptera exiqua

This species was introduced this year for routine breeding in the insectary. The colony was maintained mainly on maize leaves and, as an alternative, sweet potato leaves. This species failed to do well on an artificial diet used for *S. exempta*. However, after a number of generations, towards the end of the year, the adults were not able to

lay fertile eggs and when the trial and error methods used for other species also failed the colony was gradually lost.

Breeding of ticks

Beside the routine breeding of the soft tick *Ornithodoros moubata* this insectary also started breeding a hard tick *Rhipicephalus appendiculatus* from October 1977.

Ornithodoros moubata

This colony did well during the first six months of the year and in April had a good colony comprising about 10,000 third instar, 5,700 fourth instar and 3,200 fifth instar nymphs and an adult population of 700 females and 1,120 males.

The supply of rabbits and pig blood was stopped from VRO, Muguga, from mid-June, because of an outbreak of rinderpest. This had a very bad effect on the colony. Due to the shortage of pig blood, samples of pig blood were brought from upland farms as a substitute. For reasons unknown, these blood samples had a disastrous effect on the tick colony and more than half of the animals which were fed on them died. There was a sharp fall in the number of different instars and adults and the numbers for September were 2,060 third instar, 754 fourth instar and 200 fifth instar nymphs, with 233 females and 675 males. The colony recovered to some extent in the month of December. The department has now bought two pigs for breeding.

Rhipicephalus appendiculatus

Routine breeding of this hard tick was started in October this year and made very steady progress till the end of the year. The colony was started with 700 males and 800 females in October and the numbers for January 1978 are 1,100 males and 4,075 females.

Animal Breeding Unit

As indicated in the previous Annual Report two extra rooms were added to the Animal Breeding Unit by the middle of the year. These rooms are now being used as a rabbit breeding room and a weaning room for young rabbits.

Rabbits

A new rabbit colony was started at the beginning of the year with 8 females and 2 males. These gave birth to a total number of 69 litters during the first quarter of the year of which 46 were weaned to adults. During the second quarter of the year the numbers of breeding stock were increased to 15 females and 6 males and 118 litters were produced. Out of these, 98 were weaned to adults. During the third quarter, the breeding stock was increased to 20 females and 6 males these giving birth to 112 litters, from which 97 were weaned to adults. During the last quarter, the breeding stock was increased to 30 females and 7 males which produced 130 litters, from which 113 were weaned to adults. This section supplied 351 rabbits for use in different experiments and routine

Table 1. Summary of performance of *Glossina morsitans* colony in 1977

Months	New emergence		Average No. of mated females in the colony/ day	Mated female death	Old females removed from colony	Mated females added to the colony	Flies used in experiments	
	Male	Female					Male	Female
January	4976	4720	4657.35	1816	—	2040	907	1447
February	4102	3561	4644.25	1624	190	1880	965	954
March	3734	3869	5094.19	1189	—	1700	636	603
April	3992	3853	5786.34	1270	—	2054	609	643
May	3777	3894	5839.48	1018	368	2145	1014	583
June	4132	3845	5149.73	1298	679	1990	480	709
July	4040	3802	5470.96	1237	—	1575	1512	1243
August	2777	2827	6518.12	1675	234	2321	450	182
September	2780	2827	6711.36	1072	55	1985	1095	664
October	3302	3388	5194.45	697	284	1910	780	922
November	2480	2209	4625.80	849	522	1110	632	512
December	3238	3712	5427.06	727	—	2230	214	44

tsetse feeding, but 596 others had still to be bought from outside. Work is still going on to increase the breeding stock of rabbits so that the majority of rabbits can be supplied from the Animal Breeding Unit instead of being bought from outside.

Guineapigs

A small colony of guineapigs was maintained till the middle of the year. It was found that nobody was using these animals. Subsequently, it was decided to discontinue its breeding and most of the animals were distributed to different schools.

Rats

The rat colony did well during this year. In all, 825 rats were distributed for experimental work and some others were given to different schools.

Mice

The mice colony performed very well during the year. About 5,000 mice were produced in the year, most of which were used in different experiments.

Outside Support

Besides maintaining internal supplies, this Unit also provided support to a number of outside organizations in different ways. The Departments of Zoology, Entomology and Biochemistry of the University of Nairobi received regular supplies of tsetse flies and armyworms as teaching material.

Secondary schools from different parts of Kenya received tsetse flies, guineapigs, rats and mice. Egerton College received supplies of armyworms.

Armyworms in the pupal stages were also sent overseas. ILRAD received a regular supply of a large number of tsetse pupae. The Centre also received a regular supply of tsetse pupae from the U.K. and France.

Training

During the year the Tsetse Insectary took part in training two Somali women in the breeding and maintaining of tsetse flies.

Table 2. Summary of fecundity, fertility and female mortality of *Glossina morsitans* colony in 1977

Months	Total No. of pupae collected	Average production per female/month	Total numbers weighed	Total weight gm	Mean weight in m gm	Rate of female mortality (%)	Pupae given out for experiments
January	9804	2.1	1706	50.3131	29.5	1.3	62
February	8106	1.8	1585	44.2861	27.9	1.3	80
March	9408	1.9	2011	59.3390	29.5	0.8	318
April	9578	1.8	1872	50.5166	27.0	0.7	280
May	10281	1.8	2095	57.1426	27.3	0.5	305
June	8783	1.7	1572	48.7092	31.0	0.9	275
July	6864	1.3	1392	40.0612	28.8	0.8	155
August	6560	1.1	1082	32.3260	29.9	0.9	80
September	7031	1.1	1381	41.2350	29.8	0.5	25
October	8229	1.2	1639	48.0439	29.0	0.6	60
November	7905	1.8	1418	38.2275	29.2	0.6	55
December	9655	2.1	2155	66.8715	31.0	0.5	—

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