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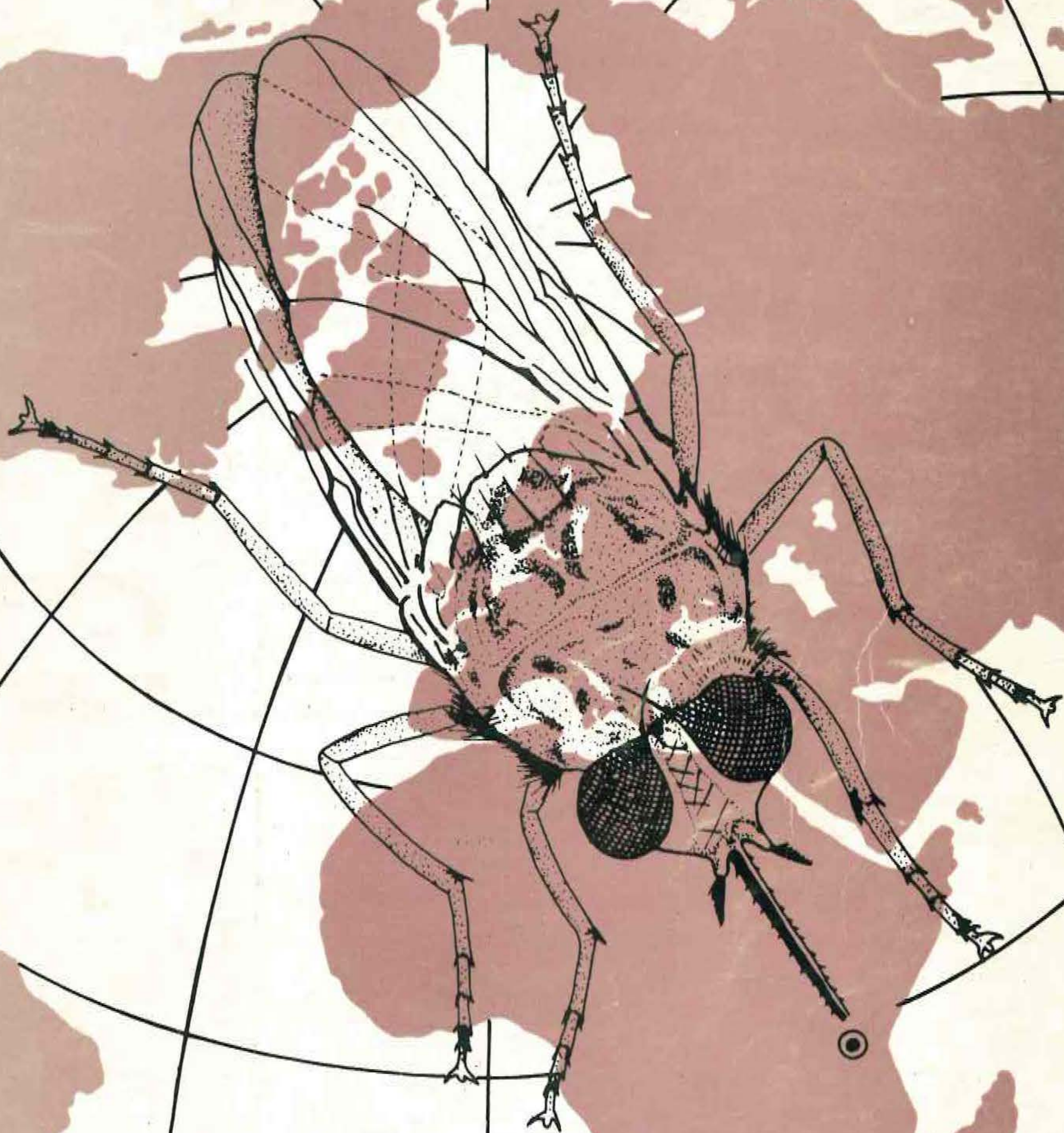

ICIPE

SEVENTH ANNUAL REPORT - 1979

Tenth Anniversary 1970-1980

THE INTERNATIONAL CENTRE OF INSECT PHYSIOLOGY AND ECOLOGY

SEVENTH ANNUAL REPORT

1979

Nairobi, March 1980

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The Royal Netherlands Academy of Science and Letters, Amsterdam, Netherlands
The Royal Society, London, UK
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The Royal Swedish Academy of Science, Stockholm, Sweden
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Professor Thomas R. Odhiambo, Director, ICIPE

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Professor J. H. Law
Dr. R. Leuthold
Dr. A. R. Njogu
Professor J. W. S. Pringle
Dr. D. J. W. Rose
Professor L. M. Schoonhoven
Dr. R. Subra

PROGRAMME LEADERS

Programme	Leader	Country	Appointed
African Armyworm Research	Dr. D. J. W. Rose (Honorary position)	United Kingdom	1.7.77
Livestock Tick Research	Dr. M. P. Cunningham	United Kingdom	1.10.77
Tsetse Research	Dr. A. Challier	France	1.9.78
Medical Vectors Research	Dr. R. Subra	France	1.11.78
Crop Borers Research	Professor E. H. Smith	USA	1.9.79
Grassland Termites Research	Professor W. L. Nutting	USA	1.9.79
Bases of Plant Resistance to Insect Attack Research	Dr. Z. T. Dabrowski (Acting Programme Leader)	Poland	10.9.79

RESEARCH SCIENTISTS

Programme / Name	Country	Appointed
African Armyworm Research		
Dr. S. Khasimuddin	India	1.12.73
Dr. B. I. P. Persson	Sweden	1. 6.77
Bases of Plant Resistance		
Dr. R. C. Saxena	India	1. 7.77
Medical Vectors Research		
Dr. J. B. Kaddu	Uganda	1.12.79
Dr. A. W. R. McCrae	United Kingdom	1.11.77
Dr. F. W. Mosha	Tanzania	1.3.78
Dr. M. J. Mutinga	Kenya	1.7.79
Sorghum Shootfly Research		
Mr. A. G. L. Delobel	France	1.10.78
Dr. A. K. Raina	India	1.9.77
Dr. G. C. Unnithan	India	1.9.78
Termite Research		
Dr. M. A. Arshad	Canada	29.6.77
Dr. J. P. E. C. Darlington	United Kingdom	1.10.74
Dr. V. D. P. Nair	India	1.9.79
Dr. G. W. Oloo	Kenya	1.5.74
Dr. T. O. Oloya	Uganda	19.11.79
Dr. D. B. A. Ruyooka	Uganda	1.9.78
Livestock Tick Research		
Dr. R. M. Newson	United Kingdom	1. 9.74
Dr. F. D. Obenchain	USA	6.10.76
Tsetse Reproductive Physiology		
Dr. M. F. B. Chaudhury	Bangladesh	1. 3.74
Dr. M. S. Ramasamy	Sri Lanka	16.8.78
Tsetse Salivary Gland Physiology		
Dr. T. K. Golder	USA	1.4.78
Dr. F. L. Lambrecht	United Kingdom	1.4.78
Dr. M. B. A. Nyindo	Tanzania	1.5.78
Dr. J. O. Olobo	Uganda	1.5.78
Dr. L. H. Otieno	Kenya	1.2.73
Dr. C. Powell	USA	6.12.79
Tsetse Population Diversity		
Dr. J. van Etten	Holland	31.1.74—31.12.79
Dr. F. W. Snow	United Kingdom	1.12.77
Dr. D. A. Turner	United Kingdom	23.11.78
Bioassay		
Dr. T. Gebreyesus	Ethiopia	14.3.78
Chemistry and Biochemistry		
Dr. P. G. McDowell	United Kingdom	1.3.79
Dr. D. A. Otieno	Kenya	23.2.79
Dr. D. L. Whitehead	United Kingdom	17.4.79
Histology and Fine Structure		
Dr. E. D. Kokwaro	Kenya	1.12.75

			Appointed
Sensory Physiology			
	Dr. J. V. Clark	United Kingdom	1.1.77
	Dr. J. H. MacFarlane	Canada	25.4.77—23.10.79
	Dr. S. Waladde	Uganda	18.9.78
Insect Mass Rearing			
	Dr. R. S. Ochieng	Kenya	1.11.77
Insect Pathology			
	Dr. G. P. Kaaya	Tanzania	1.7.78
	Dr. W. Otieno	Kenya	8.2.78

SCIENTIFIC OFFICERS

Sorghum Shootfly Research			
	Mr. K. Ogwaro	Uganda	1.9.73
Termite Research			
	Dr. B. M. Okot-Kotber	Uganda	13.2.76
Livestock Tick Research			
	Dr. G. M. Binta	Uganda	3.12.78
	Mrs. C. K. A. Mango	Kenya	1.7.71
	Mr. D. K. Punyua	Kenya	1.9.73
Tsetse Reproductive Physiology			
	Mr. T. S. Dhadialla	Kenya	1.10.73
	Mr. J. Kawooya	Uganda	1.9.73
Tsetse Salivary Gland Physiology			
	Mrs. N. Y. Patel	Kenya	1.3.75
Chemistry/Biochemistry			
	Mrs. M. A. Vundla	Kenya	1.2.75
Histology and Fine Structure			
	Mr. J. Owor	Uganda	1.12.73
Sensory Physiology			
	Mr. R. K. Saini	Kenya	2.6.76
Legume Pod-Borers Research Project			
	Mr. J. B. Okeyo-Owuor	Kenya	1.10.78

RESEARCH ASSISTANTS

African Armyworm Research			
	Mr. B. L. Otindo	Kenya	11.1.76
Livestock Tick Research			
	Mr. J. W. Chiera	Kenya	9.10.76
Tsetse Reproductive Physiology			
	Mrs. R. W. Kunyiha	Kenya	18.5.76
Tsetse Salivary Gland Physiology			
	Miss N. F. Darji	Kenya	1.10.74
Histology and Fine Structure Research Unit			
	Mrs. J. A. Kongoro	Kenya	16.4.74

TECHNICAL STAFF

			Appointed
African Armyworm Research			
Mr. J. T. Kilori	Technician	Kenya	6.11.72
Mr. G. M. Kinyanjui	Technical Assistant/Driver	Kenya	11.3.77
Mr. M. Lubega	Junior Technician	Uganda	1.3.74
Mr. D. M. Mathenge	Junior Technician	Kenya	1.3.74
Mr. G. M. Nganga	Subordinate Assistant	Kenya	15.2.74
Mr. R. Okello	Technical Assistant	Kenya	1.3.73
Mr. C. Were	Technical Assistant	Kenya	1.1.77
Medical Vectors Research			
Mr. P. Amutalla	Technical Assistant	Kenya	1.3.79
Mr. E. Mkuzi	Technical Assistant	Kenya	1.6.76
Mr. S. Muti	Junior Technician	Kenya	1.3.78
Mr. P. Mwamisi	Junior Technician	Kenya	1.3.78
Mr. J. Mwandandu	Technical Assistant/Driver	Kenya	1.10.71
Mrs. N. M. Ouna	Principal Technician	Australia	1.9.79
Termite Research			
Mr. P. O. Amoke	Technical Assistant	Kenya	1.9.79
Mr. L. Busharizi	Senior Technician	Uganda	1.10.77
Mrs. M. N. Baraza	Technician	Kenya	1.10.74
Mr. M. O. Kotengo	Junior Technician	Kenya	1.1.79
Mr. S. L. Montet	Technical Assistant/Driver	Kenya	1.12.78-31.12.79
Mr. E. Nyandat	Junior Technician	Kenya	1.10.77
Miss M. G. Wanjiru	Technical Assistant	Kenya	1.12.75
Livestock Tick Research			
Mr. A. Bwire	Technical Assistant	Kenya	1.9.75
Mr. G. M. Hindi	Subordinate Assistant	Kenya	1.3.74
Mr. J. G. Mugane	Technical Assistant	Kenya	1.8.73
Mr. J. N. Ndungu	Subordinate Assistant	Kenya	1.8.73
Mr. R. Ojowa	Junior Technician	Kenya	15.2.75
Mr. F. M. Thuo	Technical Assistant	Kenya	14.6.77
Mr. K. C. Wainaina	Subordinate Assistant	Kenya	1.3.74
Tsetse Reproductive Physiology			
Mr. F. Mukunza	Junior Technician	Kenya	14.11.73
Mr. P. Osula	Junior Technician	Kenya	1.3.78
Tsetse Salivary Gland Physiology			
Miss R. Chesang	Junior Technician	Kenya	1.3.72
Mr. J. Likhanga	Technical Assistant/Driver	Kenya	1.11.74
Mr. E. Mpanga	Technician	Uganda	1.3.78
Mr. P. Onyango	Technician	Kenya	1.12.74
Tsetse Population Diversity			
Mr. J. O. Apale	Technician	Kenya	1.5.76
Mr. F. Kathuli	Technical Assistant/Driver	Kenya	1.1.77
Mr. J. Kiilu	Subordinate Assistant	Kenya	7.6.76
Mr. A. J. Makau	Technician	Kenya	1.6.76
Mr. D. K. Mungai	Technical Assistant/Driver	Kenya	1.6.78
Mr. R. Mutuaruhiu	Junior Technician	Kenya	1.9.72
Mr. D. F. Uvyu	Junior Technician	Kenya	1.12.74
Bioassay			
Mr. G. Achieng	Junior Technician	Kenya	1.9.76
Mr. L. Moreka	Technical Assistant	Kenya	1.9.76
Mr. B. N. Odero	Principal Technician	Kenya	21.10.76
Mr. E. N. Ole Sitayo	Senior Technician	Tanzania	1.4.79

Chemistry and Biochemistry			Appointed
Mr. A. Chapya	Principal Technician	Kenya	16.4.74
Mr. N. Juma	Senior Technician	Kenya	1.4.74
Histology and Fine Structure			
Mr. M. Chimtawi	Principal Technician	Tanzania	15.1.74
Mr. P. Lisamulla	Senior Technician	Kenya	1.2.73
Mrs. J. Muriithi	Senior Technician	Kenya	1.5.79
Mr. N. T. Ogoma	Junior Technician	Kenya	13.7.79
Sensory Physiology			
Mr. H. M. Kahoro	Technician	Kenya	1.5.75
Insect and Animal Breeding			
Mr. J. Atema	Junior Technician	Kenya	1.10.75
Mr. E. Awuoche	Technical Assistant	Kenya	1.12.73
Mr. H. Banda	Technician	Kenya	16.2.72
Mr. G. M. Birir	Technical Assistant/Driver	Kenya	1.2.78
Mr. A. Ikhunyalo	Junior Technician	Kenya	16.2.71
Mr. J. Kagoiya	Technician	Kenya	1.10.73
Mrs. R. Kariuki	Technical Assistant	Kenya	1.10.74
Mr. J. Ongudha	Technician	Kenya	1.10.73
Mr. J. Wanyonje	Senior Technician	Kenya	1.6.70
Sorghum Shootfly Research			
Mr. G. O. Amala	Technical Assistant	Kenya	1.5.76
Mr. G. M. N. Bizoza	Principal Technician	Uganda	15.9.77–31.12.79
Mr. K. E. Kidega	Senior Technician	Uganda	1.10.77
Mr. J. D. B. Limuli	Technical Assistant/Driver	Kenya	2.1.79
Mr. J. C. Olela	Senior Technician	Kenya	30.9.77
Mr. S. M. Othieno	Technician	Kenya	1.4.74
Bases of Plant Resistance			
Mr. J. B. Kibuka	Technical Assistant	Kenya	1.3.78
Mr. E. O. Nyangiri	Technician	Kenya	1.7.79
Mr. P. O. Odinga	Junior Technician	Kenya	1.3.78
Mr. S. O. H. Okech	Technician	Kenya	1.8.77
Mr. F. O. Onyango	Technician	Kenya	1.6.79
Miss A. A. Ragot	Technical Assistant	Kenya	25.7.77
Workshop			
Mr. A. Mando	Controller for Technical Services	Cameroon	1.3.73
Mr. H. Gichinga	Junior Technician	Kenya	1.3.76
Mr. S. Karanja	Senior Technician	Kenya	1.9.79
Mr. M. A. Lobo	Electronics Engineer	United Kingdom	15.11.79
Mr. J. M. Maina	Technician	Kenya	1.3.75
Mr. J. N. Mtei	Junior Technician	Tanzania	12.10.76
Mr. P. O. Nyachieo	Senior Technician	Kenya	1.12.73
Mr. J. N. Omondi	Technical Assistant	Kenya	12.7.74
Mr. J. B. Omulo	Junior Technician	Kenya	3.9.73
Laboratory Management			
Mr. E. E. Muro	Engineer and Controller for Laboratory Services	Tanzania	1.9.79
Communications and Information			
Mr. J. F. Shikhubari	Technician	Kenya	15.8.75

Estates and Maintenance Services

Name	Position	Country	Appointed
Mr. S. Akhaya	Assistant Janitor	Kenya	16.9.74
Mr. J. Atiche	Janitor	Kenya	7.1.74
Mr. J. N. Musisi	Maintenance Engineer	Uganda	29.1.79
Mr. J. O. Onyango	Plumber/Mason	Kenya	1.2.78

FIELD STATIONS

Mbita Point Field Station

Name	Position	Country	Appointed
Mr. J. O. Angado	Maintenance Engineer	Kenya	1.10.79
Mr. B. S. K. Masyanga	Farm Controller	Kenya	25.11.77
Mr. P. Mbuya	Technical Assistant/Driver	Kenya	1.11.77
Mrs. M. N. Okach	Assistant Secretary	Kenya	9.1.78
Mr. E. Omolo	Agronomist	Kenya	3.4.78
Mr. J. O. Omuodo	Administrative Officer	Kenya	16.1.78
Mr. F. K. Ongola	Assistant Accountant	Kenya	1.1.79

Coastal Field Station

Name	Position	Country	Appointed
Mr. J. B. Kagoh	Technical Assistant/Driver	Kenya	13.11.78
Mr. M. M. Moinde	Administrative Officer	Kenya	1.11.78
Miss L. G. Munge	Copy Typist	Kenya	1.9.77
Mr. P. O. Ngugi	Senior Accounts Clerk	Kenya	1.1.79
Mrs. C. M. Rarieya	Assistant Secretary	Kenya	7.12.78
Mr. E. M. Sowah	Technical Assistant/Driver	Kenya	1.3.78

SENIOR MANAGEMENT STAFF

Position	Name
Director	Professor Thomas R. Odhiambo
Manager for Communication Systems	Mr. J. M. Ojal
Deputy Director (Research)	Professor A. S. Tahori
Administrative Manager	Mr. C. O. Angoma
Financial Manager	Mr. L. Z. Mosha

ADMINISTRATIVE STAFF

Office of the Director

Name	Position
Miss D. T. Adhiambo	Administrative Secretary
Mrs. M. U. Arara	Personal Assistant
Miss M. H. Bugembe	Secretary
Miss R. J. Chesum	Assistant Secretary
Mrs. R. A. Odingo	Senior Planning Officer
Mr. W. O. Ogalo	Planning Officer
Miss M. Wafula	Secretary

Communications and Training Division

Name	Position
Dr. L. O. Abe	Senior Training Officer
Mr. C. Bwire	Sculptor/Artist
Mr. M. A. Foum	Communications Officer
Miss H. N. Githinji	Typesetter
Miss E. N. Kahuhu	Library Assistant
Mr. D. R. Kigera	Librarian
Miss F. Ojode	Secretary
Mrs. R. P. Ortega	Documentalist
Mrs. A. W. Oyuko	Scientific Illustrator
Miss R. Washika	Training Officer

Office of the Deputy Director (Research)

Name	Position
Miss R. Runo	Secretary

Personnel and Office Management

Name	Position
Miss E. Afandi	Assistant Secretary
Mrs. M. Antao	Assistant Secretary
Mr. S. M. Kimaita	Administrative Officer
Mrs. E. P. Kwach	Telephonist/Receptionist
Mrs. T. Lohay	Secretary
Mr. J. E. Okiri	Administrative Officer
Mrs. R. Okoth	Assistant Secretary
Mrs. A. A. Okumali	Secretary
Mrs. P. Owitti	Secretary
Miss R. Vugaba	Secretary
Mrs. G. Weya	Senior Telephonist/ Receptionist

Finance Department

Name	Position
Mr. G. W. Kanza	Expenditure Accountant
Mr. N. Kiongo	Assistant Accountant
Mr. J. K. Kitur	Accounts Clerk
Mr. M. P. Macohito	Supplies Assistant
Mr. B. Mwangi	Assistant Accountant
Mr. A. A. Oguda	Assistant Accountant
Mr. R. M. P. Okura	Accountant
Mrs. R. Opande	Secretary

Trainees

Name	Position
Mr. T. S. Dhadialla	Scientific Officer
Miss Lucy Irungu	Graduate Research Scholar
Mr. J. I. Jondiko	Graduate Research Scholar
Dr. G. P. Kaaya	Postdoctoral Research Fellow
Mr. J. Kawooya	Associate Scientific Officer
Mr. A. Mongi	Graduate Trainee
Dr. V. P. Nair	Postdoctoral Research Fellow
Mr. J. J. Njokah	Graduate Research Scholar
Mr. G. N. H. Nyamasyo	Graduate Research Scholar
Mr. J. Nyoike	Technical Assistant Trainee
Mr. W. O. Ogalo	Planning Officer
Mr. S. H. Okech	Senior Technician
Miss Lucy Oketch	Graduate Research Scholar
Mr. R. M. P. Okura	Accountant
Mr. J. C. Olela	Senior Technician
Dr. J. O. Olobo	Graduate Research Schola
Dr. T. O. Oloya	Postdoctoral Research Fellow
Mr. Patrick Oluya	Technical Assistant Trainee
Miss D. Sabwa	Graduate Research Scholar
Mr. J. G. Yarro	Graduate Trainee

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RESEARCH STRATEGY AND PRIORITIES

Over the last 18 months, the International Centre of Insect Physiology and Ecology (ICIPE) has devoted considerable time and effort into reviewing the long-term objectives of the ICIPE core research programmes and the manner in which to attain them—an exercise which ended in the compilation of a well thought out document termed "The ICIPE Strategic Research Plans". The institute's Research Committee was intimately involved in this task, and the Governing Board was advised on the proposals put forward by the Committee by a number of ICIPE's advisory bodies (the African Committee, the Policy Advisory Committee, and the Research and Training Council). The most important result of the review excercise was the consolidation of the ICIPE research effort into 7 core programmes and 4 research units, which the Board accepted early in 1979:

- Bases of Plant Resistance to Insect Attack
- Crop Borers (as it relates to cereals, legumes, and sugarcane)
- African Armyworm
- Grassland Termites (in the semi-arid savannah ecosystem)
- Livestock Ticks (in relation to East Coast Fever and related animal diseases)
- Tsetse
- Medical Vectors (of malaria, bancrofti filariasis, and leishmaniasis)
- Research Units on Chemistry and Biochemistry, Histology and Fine Sctructure, Sensory Physiology, and Bioassay.

This consolidation of ICIPE's programmes was alluded to in the ICIPE's *Sixth Annual Report* and has led to sharper resolution of ICIPE's focus on the major pest management questions that need answering prior to the evolution of long-range, environmentally acceptable integrated pest and vector management systems.

The multi-disciplinary scientific strength represented in the ICIPE—which ranges from natural products chemistry to sensory physiology, from endocrinology to comparative behaviour—was given a wider perspective from the beginning of the year by the considerable strengthening of scientific staff with wide experience in ecological research. At the same time, staff with training in agronomy, epidemiology, and insect pathology were recruited as part of the target insect-oriented research programmes. This array of research approaches has deepened ICIPE's potential for extensive exploration of transdisciplinary areas, for linking fundamental scientific knowledge with actual field problems, and the articulation of scientific discoveries with development problems. Indeed, the transformation of the old practice of Entomology to a new science of "Insect Science" has become a reality at the ICIPE.

Early in 1978, the ICIPE—on the advice of the ICIPE Foundation—appointed a Visiting Group on Administration and Finance. The Visiting Group reported in October, 1978. The Governing Board considered the recommendations of the Visiting Group, and made decisions on the governance and institutional arrangements of the ICIPE at its 35th Meeting held in January 1979—after having consulted the ICIPE Foundation. The principal decisions of the Board were that the system of appointing non-resident but world-renowned scientists as Visiting Directors of Research to visit the ICIPE programmes frequently and guide its work be phased out by June 1979, and that instead the ICIPE appoints into its staff cadre senior scientists as Programme Leaders. It was further envisaged that short-term consultancies for specific areas of methodology or research concern be strengthened. Furthermore, the Board decided to phase out the many advisory bodies that the institute has acquired over the years, and instead institute a committee structure in the Board to reflect the policy needs in respect of programming, finance and administration, and Board composition. Three committees were established, and have been in operation since June 1979—Executive Committee (which deals with all matters in between full Board meetings, and also deals with finance business), Nominating Committee (which considers new nominations for the Board) and Programme Committee (which is especially interested in research and training policies of the Centre). The rationalised system of the governance of the ICIPE corresponds closely to the effort which culminated in 1979 in having a lean and sharply focussed scientific programme.

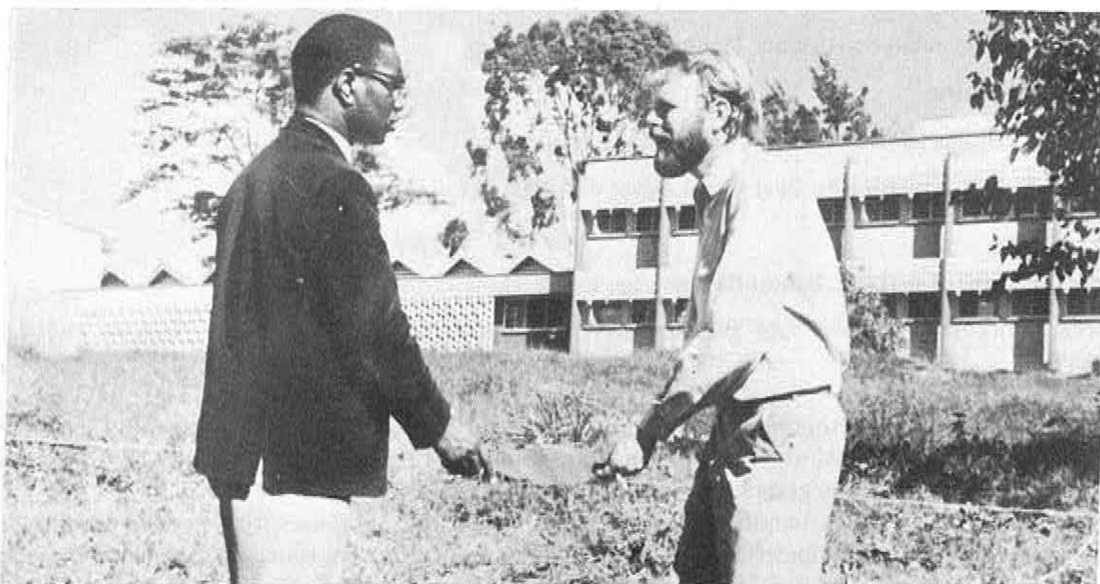
At the time of writing this Introduction to the ICIPE 7th Annual Report, the Centre is approaching its 10th Anniver-

sary since it was legally established under Kenyan Law on 7th April 1970. We look forward to the year 1980 proving a watershed for scientific achievements in ICIPE's programmes, as 1970 proved to be the source of a new model of institutional development for research establishments in development-oriented research.

Thomas R. Odhiambo

Director, ICIPE

14th March 1980.



ICIPE 1970 — 1980: The photograph above shows the Director, Professor Thomas R. Odhiambo discussing the plans for the first building for the Centre. Below is seen the ICIPE International Guest Centre at Duduville.

TRAINING AT THE ICIPE 1979

During 1979, the ICIPE supported or conducted a total of 41.89 man-years of training (See Table 1). These comprised formal training at ICIPE for its own staff and others from various institutions, or sponsorship of its own staff for training in other institutions. No new training programmes were initiated.

Table 1. Training output at the ICIPE, 1979

Type of Training	No. of Man-years
1. High School Bursars Scheme	5.0
2. Graduate Training	
. ICIPE Staff at ICIPE	
MSc	2.0
PhD	2.0
. ICIPE Staff in other institutions	
MSc	2.5
PhD	2.0
. At ICIPE for other institutions	
MSc	1.0
PhD	2.0
3. Graduate Research Scholarship	3.0
4. Research Associateship	0.25
5. Specialised Training	
. At ICIPE for other institutions	2.25
. ICIPE staff in other institutions	3.33
6. Postdoctoral Training	3.66
7. Technical Training (at Kenya Polytechnic)	5.00
8. Management Training	1.60
9. Secretarial Training	0.75
10. International Group Training Course in Pest Management Systems	1.55
11. ICIPE French Course	4.00
TOTAL	41.89

The Centre concentrated its efforts on consolidating and rationalising the existing and on-going programmes.

(1) High School Bursars Scheme

Scientific motivational training under the High School Bursars scheme continued to attract pre-university students.

(2) Graduate Training

Graduate training, the major hub of the Centre's emphasis for high-calible research capability building continued for ICIPE staff and those from other developing countries. The following undertook training through ICIPE:

- Mr. J. G. Yarro, from the University of Dar-es-Salaam continued with his PhD.
- Miss H. Thindwa, from Malawi, registered for MSc. degree at the University of Nairobi also continued
- Miss E. Opiyo, from Kenya Trypanosomiasis Research Institute continued her training.
- Miss Lucy Oketch and Miss Lucy Irungu successfully completed their studies at the Liverpool School of Tropical Medicine and will return to ICIPE to work as graduate research scholars in 1980.
- Mr. J. Kawooya continued into his third year of PhD work at the University of Illinois, Urbana, U.S.A.
- Mr. T. S. Dhadialla proceeded to Queens University, Kingston, Canada to embark on PhD studies in insect physiology and biochemistry.
- Mr. B. M. Okot-Kotber presented his PhD dissertation at the University of Dijon, France.
- Mr. Suleman Okech, having completed his technical training at IRRI on rice brown planthopper, proceeded to the University of Philippines at Los Banos to undertake MSc studies.
- Miss Diana Sabwa, Mr. J. O. Jondiko, and Mr. Nyamasyo joined ICIPE as graduate research scholars in insect pathology, natural products chemistry and termite ecology, respectively.
- Mr. A. O. Mongi continued his PhD studies at the University of Nairobi.
- Mr. K. Ogwaro presented his PhD dissertation at the University of Nairobi.
- Dr. (Mrs.) C. K. A. Mango completed her PhD work at the University of Nairobi.
- Mr. J. Mwega continued his MSc course-work at the University of Nairobi, with research work at ICIPE.

(3) Research Associateship

- Dr. Adebayo Odebiyi, from the University of Ibadan undertook a further 3-month study at ICIPE.

(4) Specialised Training

- Mrs. Khitma E. El Malik, an Andre' Mayer Research Fellow/FAO worked at ICIPE from January; attended the ICIPE International Group Training Course in Pest and Vector Management; and proceeded to work on the ecological and epidemiological field studies of tsetse flies.

Mr. C. M. Mutero, postgraduate student from the University of Nairobi continued his studies on mosquito ecology.

Dr. Arona Gueye, from Institut Senegalais de Recherche Agricole, Dakar, Senegal spent 3 months (April-July) studying research techniques in tick physiology and ecological field studies at the ICIPE. He was sponsored by the ICIPE and ORSTOM, France.

- Mr. P. Oluya and Mr. J. Nyoike are undergoing 6-month training in glass blowing, fabrication and repair of laboratory glassware at the Tata Institute of Fundamental Research, Bombay, India. They will complete their training early 1980.
- Dr. Joseph Buruga, Botany Department, Makerere University, Uganda, spent 2 months (August–September) studying tsetse breeding techniques and some aspects of gel electrophoresis.

(5) Postdoctoral Training

This has continued to attract young scientists from developing countries.

- Dr. (Mrs.) V. P. Nair, from India joined ICIPE to work on soil chemistry in the Termite Research Programme.
- Dr. T. O. Oloya, Ugandan, recently completed his PhD at Ohio State University on soil chemistry and fertility has also joined the Termite Research Programme. He will be proceeding to Australia in March 1980 for a 9-month research training in the area of effect of termite on soils under the supervision of Dr. K. E. Lee of CSIRO.

(6) Technical Training

This has progressed in the area of biological laboratory technology and other advanced technical areas where in-house training at ICIPE is inadequate. This has been limited to the Kenya Polytechnic.

(7) Management and Secretarial Training

Training for the administrative cadre has been instituted, delving in the areas of research management and upgrading of skills of secretaries to accord it more efficiency.

(8.) The International Group Training Course in Pest Management Systems

The third course in the series was conducted from 15th July to 4th August 1979. A total of 27 participants attended this course as follows: Colombia (1), Ethiopia (2), Ghana (2), Kenya (6), Mauritius (1), Nigeria, (3), Pakistan (1), Philippines (2), Saudi Arabia (1), Sudan (2), Tanzania (2), Uganda (4).

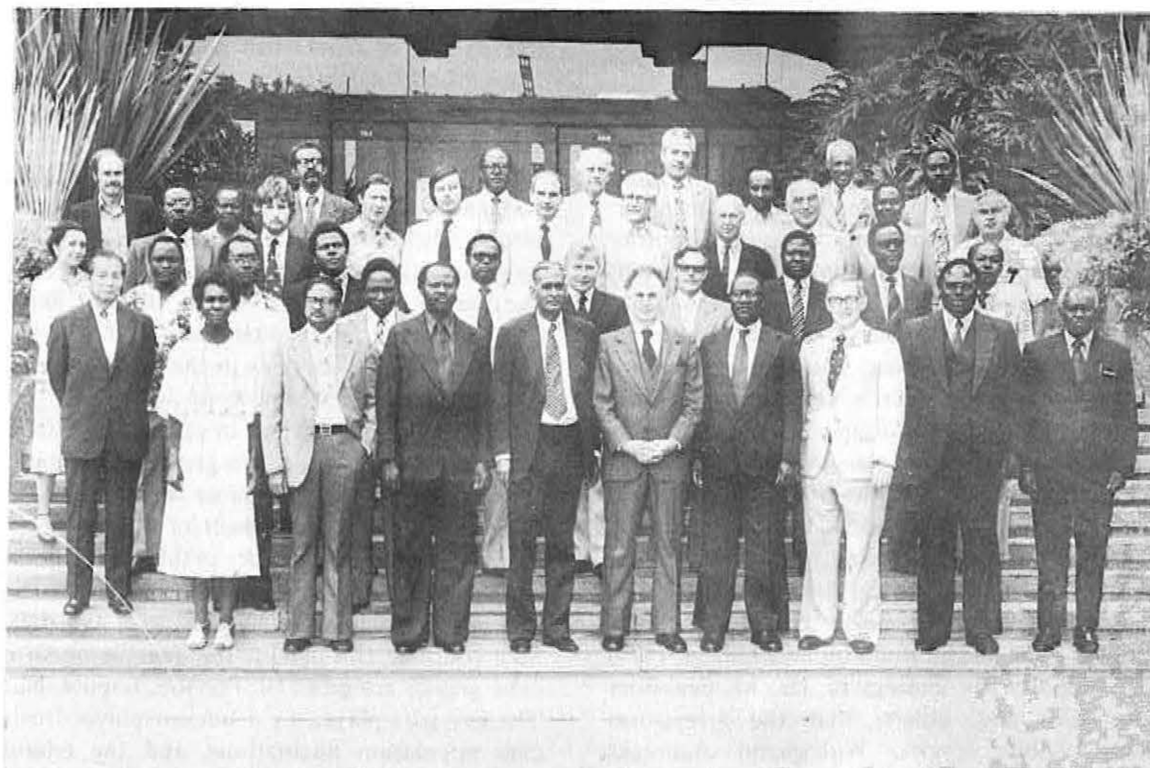
This brings the total for the third year running to 74. The course has proved extremely popular with young practitioners in pest management and ICIPE intends to consolidate and strengthen its input.

Participants to the ICIPE International Group Training Course in Pest and Vector Management receiving instructions on medical vector management while on a visit to Mwea Irrigation Settlement Scheme.

(9) International Study Workshops

Two important study workshops were held at ICIPE in 1979, these were: The Epidemiology of African Trypanosomiasis in April co-sponsored by the ICIPE and USAID, and the International Technical Workshop on Appropriate Industrial Technology for the Control of Tropical Insect Pests and Disease vectors, from 29th July to 5th August, co-sponsored by the ICIPE and the United Nations Industrial Development Organisation (UNIDO).

The former was attended by a total of 30 scientists comprising experts from Australia, Congo, England, France, Israel, Italy, Kenya, Mali, Zimbabwe, Scotland, Switzerland, Tanzania, Zaire and Zambia. Participants for the technical workshop were from: Brazil, Chile, Denmark, England, Ghana, India, Ivory Coast, Kenya, Malawi, Nigeria, Switzerland, Tanzania, Uganda, USA and Zambia.



Participants to the International Technical Workshop on Appropriate Industrial Technology for the Control of Tropical Insect Pests and Disease Vectors.

Those in the photograph include Dr. Abd-El Rahman Khane, Executive Director UNIDO (Front row, 5th right) and the Director of ICIPE, Professor Thomas R. Odhiambo (Front row, 4th right).

Also in the photograph are Mr. W. A. J. Okumu (Front row, 2nd right), Chief, Section for Economic Co-operation among Developing Countries, UNIDO and Professor W. S. Bowers who chaired the Workshop (Font row, 3rd right).

AFRICAN ARMYWORM RESEARCH

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

Dr. S. Khasimuddin (1973) Research Scientist
Dr. B. I. P. Persson (1977) Research Scientist
Mr. J. T. Kilori (1972) Technician
Mr. M. Lubega (1974) Junior Technician
Mr. D. N. Mathenge (1973) Junior Technician
Mr. G. N. Mburu (1974) Subordinate Assistant
Mr. R. Okello (1973) Technical Assistant
Mr. B. L. Otindo (1975) Associate Scientific Officer
Mr. C. Were (1977) Technical Assistant
Mr. J. Yarro (1977) Graduate Trainee

The African armyworm programme

Introduction

D. J. W. Rose

The discovery that moth flight durations are partly determined by the extent to which armyworm caterpillars are crowded during development (A. G. Gatehouse, unpubl. manuscript), has brought together research findings which sometimes appeared contradictory, with groups of scientists seeming to favour alternative hypotheses about the sources of moths. Some scientists believed that outbreaks were due to the build up of local populations, and others that they were caused by immigration. Dr. Gatehouse has shown that the crowded caterpillars found in outbreaks in the gregarious phase produce the greatest proportion of long fliers and that most moths from solitary phase do not fly far. His discovery supports Dr. Khasimuddin's field observations that moths could be divided into flitters, short and long distance fliers Khasimuddin, 1980 (in press) and that successive generations occur in the Lambwe Valley (Khasimuddin and Lubega 1979) and it also supports the findings of Dr. M. den Boer (den Boer, 1978) and others, that the armyworm is a highly mobile species. Widespread outbreaks in Kenya during 1979 provided opportunities for field studies of the behaviour of moths at outbreaks; and these studies indicated that many moths fly into nearby trees shortly after emergence from pupae in the ground and congregate in large numbers from about midnight to 5 a.m. before flight. Most moths seem to fly downwind at dusk and before dawn, and many shelter during the day under grass tufts and bark of trees (D. Rose and C. Dewhurst, in press). Observations by radar of moth flight immediately after emergence are being analysed. (J. Riley and D. Reynolds, pers. obs.).

It has been suspected that moths are concentrated by wind patterns and moth behaviour before oviposition. An opportunity to check this fact occurred when meteorologists located a region of wind convergence about 30 km downwind from a site where moths were emerging from the ground (M. Tucker and D. Pedgley, unpubl. manuscript).

The region of convergence was visited the following night and moths were seen mating in trees (D. Rose and C. Dewhurst, unpubl. obs.). This was the first time pairing of moths had been seen in the field, and it was of added interest as mating was only seen after midnight, the time recorded in the laboratory (S. Khasimuddin, 1978.)

Dr. Persson's finding (in this report) that moths show a clear preference in selection of oviposition sites, particularly for *Cynodon dactylon* grass, fits well with the other evidence of preference of larvae for *Cynodon dactylon* (B. Persson, J. Yarro, ICIPE Report 1977, 1978), and reports of solitary phase larvae usually being found in *Cynodon dactylon* in the field. There is growing evidence that armyworm occur during the "off-season" in low density populations in places where temperatures are suitable and grasses are green throughout the year. One strongly suspected source is the coast of southern Kenya, and the establishment of an armyworm coastal station facilitates the studies in this area. The continuous rearing experiments in large outdoor cages have shown that the coast is a most suitable area, and that breeding may continue throughout the year in much of Kenya once grasses are green (B. Persson, unpubl. manuscript) The key part played by a nuclear-polyhedrosis virus in cage population fluctuations, and the relationship of the fluctuations to rainfall and sunshine (B. Persson, in the report) may be of considerable significance to understanding years of major armyworm outbreaks; and it is hoped that in-depth studies of the virus in relation to armyworm ecology will be possible in the future.

Population dynamics study

B. Persson

The study from which some preliminary results were presented in last year's annual report was completed in the first half of 1979. It involved continuous rearing attempts of armyworm populations for one year on three

climatically different locations in Kenya: Msabaha on the Coast, Nairobi, and Mbita Point on Lake Victoria. The purpose was to investigate whether or not the climatic conditions would allow an all year round survival of the species and how variations in local weather affect development and survival.

On the coast 14 generations were completed and all produced adults. The success was moderate and evenly distributed over the year. No extreme peaks occurred. In Nairobi seven (some partly overlapping) generations were completed while one failed to produce adults. Two generations gave very high success. In Mbita Point ten generations were completed while two failed.

On all three locations there was a strong significant negative correlation between temperature and developmental time. The average developmental time in Nairobi was much longer than on the other two locations: 54.9 days against 28.8 days in Mbita Point and 23.1 days in Msabaha. The longest, 77.3 days was recorded in Nairobi in June–July–August, 1978 and the shortest 19.4 days in Msabaha in January 1979. The potential number of generations as calculated from the average developmental time for each location was for Msabaha 15.8, for Nairobi 6.6. and for Mbita Point 13.1.

On all three locations the larval stage was the longest, about half the developmental time was spent in this stage. Larvae reared on stargrass (*Cynodon dactylon*) had a longer developmental time than larvae reared on maize and mortality was significantly lower. The larvae also showed a strong preference for stargrass. Mortality was highest in the larval stage, 95.3 per cent against 26.0 in the egg stage and 22.6 in the pupal stage. In the larval stage mortality with very few exceptions was caused by a nuclear polyhedrosis virus (NPV). In all instars except in instars 3 and 4 in Mbita Point there was a positive relationship between rainfall and mortality, and a negative relationship between sunshine and mortality. In Msabaha there was also a significant negative correlation between rainfall and per cent success. Generally rainfall and sunshine had a stronger influence in Msabaha than on the other two locations.

On the whole the conditions seem to have been much more hazardous in Nairobi and Mbita Point than in Msabaha. Thus the three generations which failed to produce adults all developed during periods with persistent drought. Nairobi and Mbita Point are recognized outbreak areas while the Coast rarely has any outbreaks. The results indicate that a long term uninterrupted survival in Nairobi and Mbita Point is unlikely. On the other hand for part of the year the conditions there are optimal resulting in unrestricted population build up. On the Coast the species seem to be in better harmony with its controlling factors. Thus from a climatic point of view an all-year round survival on the Coast is possible. The study also shows that in the African armyworm the most important mortality factor is a NPV and that mortality within larval populations is weather related.

Larval behaviour and mortality

B. Persson

In January 1979 an armyworm unit was established on the Coast 40 km north of Mombasa. The purpose was to make possible all year round studies on larval behaviour and mortality in a climate where neither temperature nor humidity is a limiting factor.

Mortality and colouration in different strains of the African Armyworm.

An insectary strain, two wild strains from fresh outbreak populations, and two cross strains between the insectary strain and the wild strains, were observed for mortality and larval colouration. Under the given set of conditions mortality was significantly higher in the two wild strains than in the insectary strain.

EXPERIMENT I 23.2 - 16.3.1979

	INSECTARY STRAINS 1.							INSECTARY x WILD STRAIN							WILD STRAIN 2.						
NO LARVAE PER JAR	1	2	3	4	5	SUM	%	1	2	3	4	5	SUM	%	1	2	3	4	5	SUM	%
NO 2ND INSTAR LARVAE USED	5	20	30	40	50	145		5	10	15	20	25	75		5	20	30	40	50	145	
NO LAST INSTAR LARVAE	5	12	11	16	22	66		4	3	3	4	8	22		4	7	10	13	8	42	
GREEN	0	4	6	1	6	17	25.8	0	0	0	0	2	2	9.1	0	0	0	1	0	1	2.4
BROWN	4	4	2	1	2	13	19.7	4	2	3	1	4	14	63.6	3	1	3	0	0	7	16.6
BLACK	1	4	3	14	14	36	54.5	0	1	0	3	2	6	27.3	1	6	7	12	8	34	81.0
NO ADULTS	4	11	10	11	15	51		2	2	2	3	8	17		2	4	5	9	4	24	
MALES	3	9	2	5	8	27		1	2	2	1	6	12		1	2	3	6	2	14	
FEMALES	1	2	8	6	7	24		1	0	0	2	2	5		1	2	2	3	2	10	
% EMERGED	80.0	55.0	33.3	27.5	30.0	35.2		40.0	20.0	13.3	15.0	32.0	22.7		40.0	20.0	16.7	22.5	8.0	16.6	

1) REARED IN INSECTARY FOR TWO YEARS
2) FROM OUTBREAK NEAR THIKA

χ^2 DIFF. SUCCESS INSECTARY WILD STRAIN = 9.72, P 0.01-0.001
χ^2 DIFF NO GREEN + BROWN LARVAE IN INSECTARY AND WILD STRAIN = 12.70, $P<0.001$

EXPERIMENT II 16.3 - 8.4.1979

	INSECTARY STRAIN							INSECTARY x WILD STRAIN							WILD STRAIN 1.						
NO LARVAE PER JAR	1	2	3	4	5	SUM	%	1	2	3	4	5	SUM	%	1	2	3	4	5	SUM	%
NO 2ND INSTAR LARVAE USED	5	10	15	20	25	75.		5	10	15	20	25	75		5	10	15	20	25	75	
NO LAST INSTAR LARVAE	1	1	4	3	6	15		0	1	3	4	1	9		1	0	0	0	0	1	
GREEN	0	0	2	2	6	10	66.7	0	0	2	3	0	5	55.6	1	0	0	0	0	1	100.0
BROWN	1	1	2	1	0	5	33.3	0	1	1	1	1	4	44.4	0	0	0	0	0	0	0.0
BLACK	0	0	0	0	0	0	0.0	0	0	0	0	0	0	0.0	0	0	0	0	0	0	0.0
NO ADULTS	1	1	2	2	4	10		0	1	3	3	1	8		1	0	0	0	0	1	
MALES	1	0	2	1	2	6		0	1	2	1	1	5		1	0	0	0	0	1	
FEMALES	0	1	0	1	2	4		0	0	1	2	0	3		0	0	0	0	0	0	
% EMERGED	20.0	10.0	13.3	10.0	16.0	13.3		0.0	10.0	6.7	15.0	4.0	10.7		20.0	0.0	0.0	0.0	0.0	1.3	

1. FROM OUTBREAK NEAR ATHI RIVER

Table 1: Observations on larval mortality in different strains

Mortality in the two cross strains was higher than in the insectary strain but lower than in the wild strains. Significantly more green larvae appeared in the insectary strain than in the wild strains, while most of the larvae in the cross strains were brown and in the wild strains black. Mortality was lower among green and brown larvae than among black. The results indicate that genetic isolation promotes a selection for green solitary larvae with a higher resistance to virus than the black gregarious larvae common in outbreak populations.

The effect of sterilization of eggs and foodplants on mortality

Armyworm egg batches were sterilized by putting them for 10 minutes in a 0.1 per cent bleach solutions and then for 40 minutes in a 10 per cent formaldehyde solution. Maize leaves were sterilized by exposing them for 20 minutes on each side to a UV biocidal tube in a sterilization chamber. Whole stargrass plants were exposed to the UV light for two hours. A series of cultures where either the eggbatches or the foodplants had been sterilized were run in the open coastal insectary.

For each experiment an unsterilized culture was used as a control. The number of larvae pupating and the number of adults emerging were recorded. Survival was significantly higher in the cultures where the eggs had been sterilized than in the cultures where the foodplants had been sterilized. In both cultures the survival was significantly higher than in the untreated control. The results indicate that the NPV may be present both on the eggbatches and on the foodplants.

Mortality related larval distribution and feeding activity on maize plants

Fifth instar larvae were released on 60 cm high maize plants with five leaves in a $10 \times 10 \times 3$ m large screen netting cage and their distribution observed every second hour for two days and two nights. Eaten leaf area was calculated. The larvae showed a preference for feeding on the 2nd and 3rd leaf from the tip. On all leaves attacked the leaf area consumed was significantly higher on the horizontal part of the leaf than on the other parts.

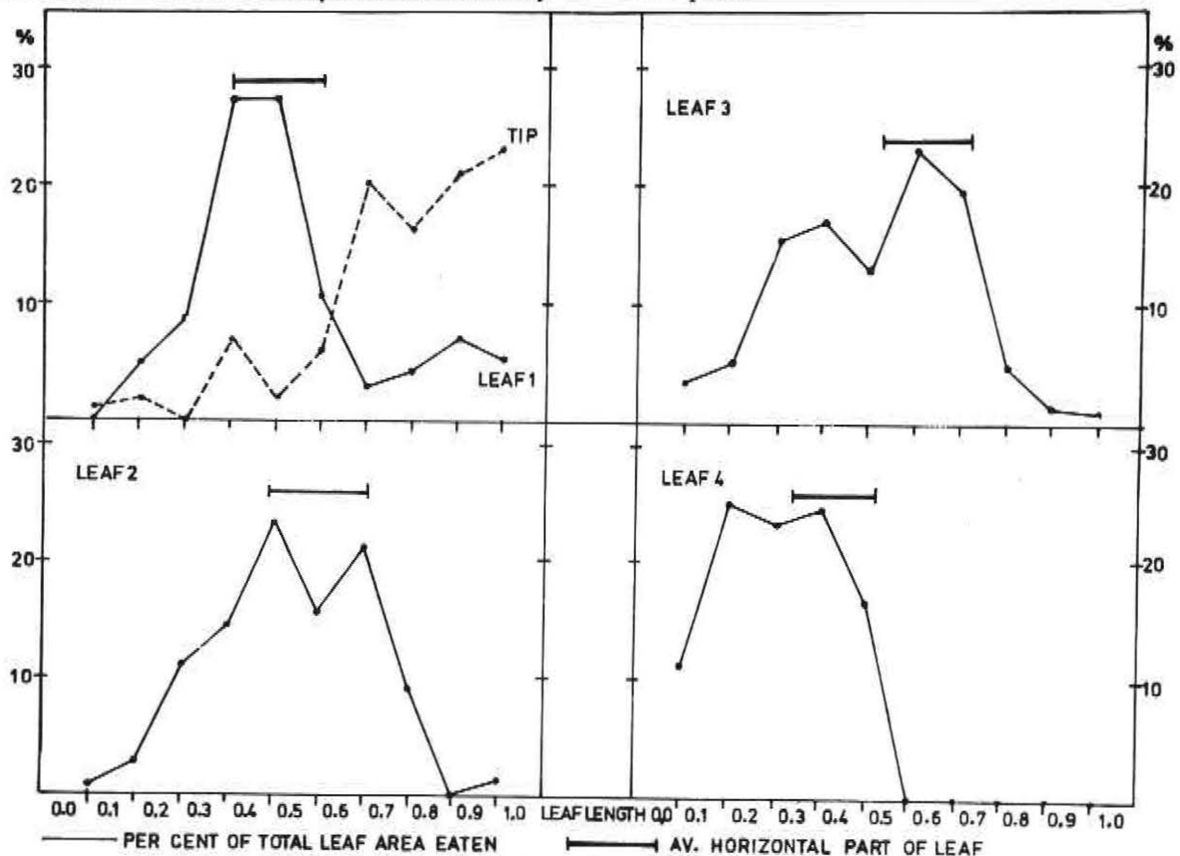


Figure 1. Distribution of feeding activity on disinfected plants of lemons.

This preference was retained even when larvae were fed leaves removed from the plants and lying flat in a box. However, if the leaves had been kept in darkness for 48 hours the preference for the in the situ horizontal area disappeared. Larvae reared on horizontal parts of leaves showed a significantly higher survival than larvae reared on base or tip parts.

The results indicate that the larvae prefer those parts of the plants which give the highest chance of survival and that the horizontal part of the upper leaves both from a nutrition point of view and possibly also from a virus point of view is the most suitable.

Armyworm Biology

B. Persson

Diurnal distribution of hatch of eggs, moulting, entry into the ground to pupate, and emergence of adults

A series of experiments were carried out where hourly observations were made for a number of days on the time of hatch of eggs, moulting, entry into the ground to pupate, and emergence of adults. The larvae were fed at irregular intervals day and night in order to avoid a phase setting effect caused by the time of feeding. The observations were carried out in a natural temperature, humidity and light regime.

Most of the eggbatches hatched in the late afternoon while most of the larvae moulted in the later half of the night, and most of the larvae entered the ground to pupate in the first part of the day.

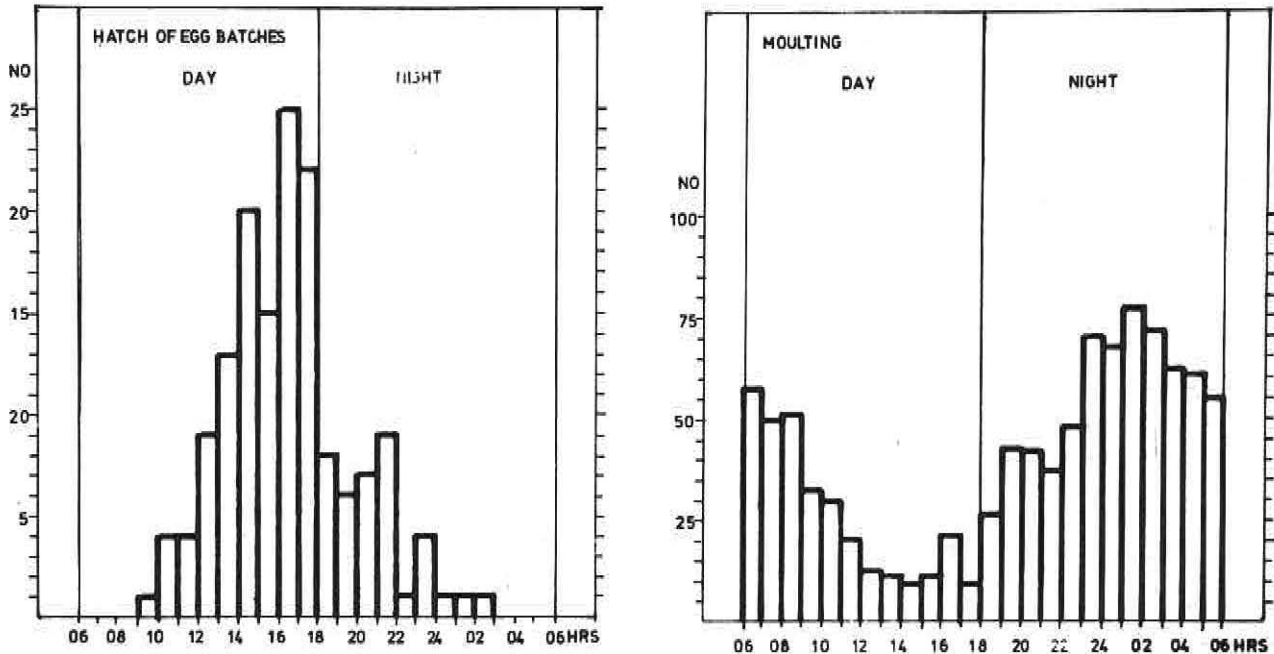


Figure 2. Hatch of eggs & moulting.

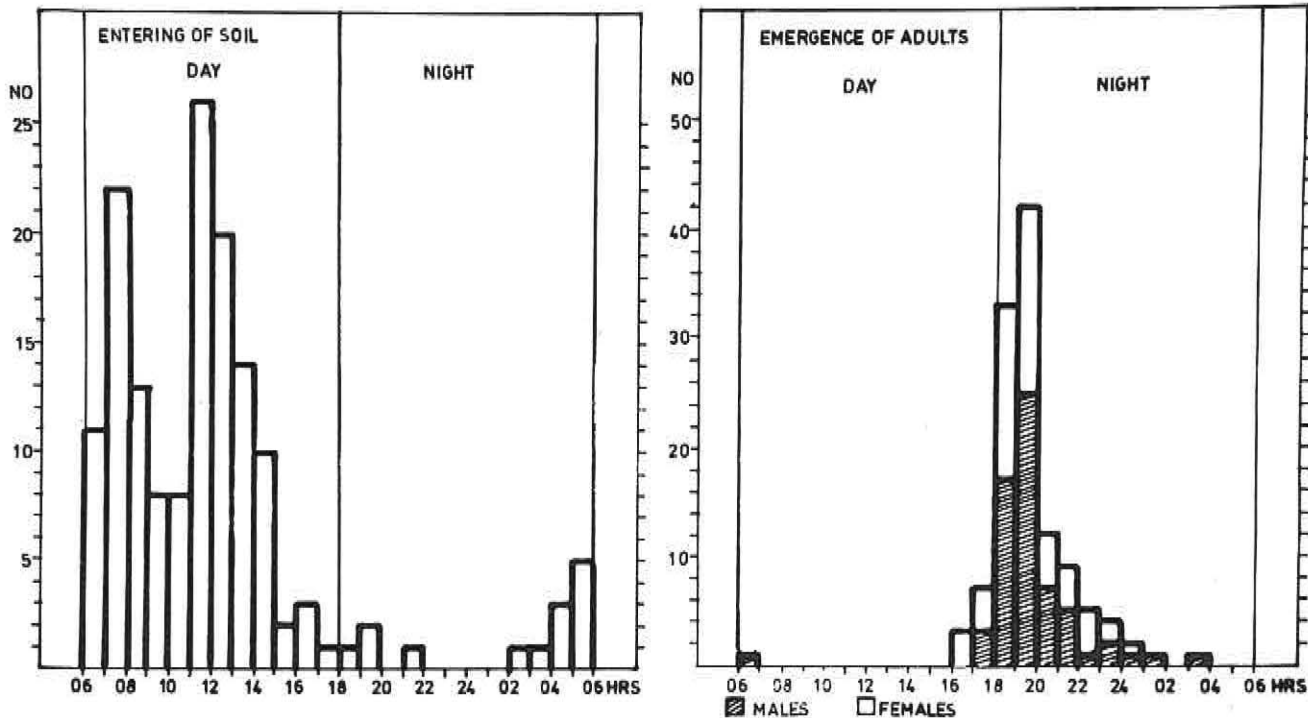


Figure 3. Entering of soil & emergence of adults.

The highest number of adults emerged in the first hours of the night. In all four cases this preference was significant. Thus the armyworm utilized the whole 24 hour cycle for these four basic activities each having its peak in its own quarter of the day and night.

Oviposition host preference

It has been believed that the armyworm female is indiscriminate in her choice of egglaying sites and that the egg-batches are laid on any suitable structure, often high up. A series of experiments carried out at the coastal armyworm unit strongly indicates that the females show a strong preference for oviposition on the host plants of the larvae. The females were offered a simultaneous choice of laying their eggs on maize, stargrass, branches, fresh leaves, various wooden structures and different wild plants. With the exception of the batch laid on a vertical branch and one laid on the lower side of a castor oil leaf, all the other batches (so far more than 100) were laid on maize or stargrass plants, with a significant preference for stargrass. It has earlier been shown that the larvae prefer stargrass over maize and that survival is higher on stargrass. In most cases the eggbatches were laid on the lower surface of one of the four uppermost leaves.

Studies on the "Off-season" survival of the armyworm, *Spodoptera exempta*.

Syed Khasimuddin

This report is of continuing research aimed at understanding the survival strategies of the insect during the "off-season". The last report (1978) presented results from investigations on effects of nutrients on developmental times, effects of different temperature regimes and phase-variation in the insect.

These studies have been developed further and some new projects initiated. The present report concentrates on the newer projects.

Effects of FARNESOL incorporation in the diet

Mansfield, et. al., (1978) suggest a build-up of farnesol in leaves of graminaceous plants as a result of the onset of drier conditions. This compound is believed to be a Juvenile Hormone (J.H.)· mimic. It was therefore considered worth testing the effect of farnesol on the development of the insect. The standard diet (Bot, 1967) referred to in the last report was used to run a pilot experiment. Two sets of larvae were reared, one on the standard diet and the other on diet containing 0.4% farnesol by weight. The farnesol incorporated in the diet was first emulsified using TRITON-X_{100} as an emulsifier. Results from this experiment are presented in Table 2. It is seen that the duration of the first instar larvae was not affected at all by farnesol. There is very little feeding during the first instar which probably explains this. However, from the second instar onwards very significantly longer times were taken by larvae fed on the farnesol diet as compared to normal diet. Significantly longer durations were also seen for the pre-pupal stages. The total duration from first instar to adult emergence was found to increase by more than 1.5 times as a result of farnesol incorporation in the diet. Larval weights taken each day revealed no significant difference between the two categories. The effect of farnesol therefore seems to be a prolongation of developmental durations. The results suggest that if farnesol in plants increases rapidly due to dry conditions, the development of the insect may be retarded considerably, which would be advantageous to the insect's strategic survival during the "off-season". These studies are being continued to include investigations in the field (field-cage) as well as more detailed investigations in the laboratory.

Table 2. Duration (days) of larval instars and pupae of *S. exempta* under normal and Farnesol treated diet

Instar	Normal ($X \pm S.E$)	Treated ($X \pm S.E$)	Significance
I	2.875±0.201	2.928±0.221	n.s
II	2.375±0.154	3.071±0.221	$P<0.02$
III	2.750±0.170	5.071±0.597	$P<0.001$
IV	2.812±0.100	3.928±0.496	$P<0.05$
V	3.437±0.364	6.538±0.895	$P<0.01$
VI	8.000±0.774	15.333±2.054	$P<0.002$
Pre-pupae	1.571±0.137	2.000±0.0	$P<0.01$
Pupae	9.571±0 368	11.500±0.499	$P<0.01$
Total duration	33.391±0.283	50.369±0.622	$P<0.001$

Comparison of J.H. titres of "Solitary" and "gregarious" larvae

In an attempt to find out if the phase-variation is hormonally controlled, titres of J.H. in larvae of both the categories were studied. These studies are in col-

Table 3. J.H. titres "solitary" Vs "gregarious" larvae at various age intervals

	J. H. titres in pg/larva	
Age	Gregarious	Solitary
00:00 hours	1.935	3.00
06:00 ,,	3.20	5.00
12:00 ,,	5.00	5.50
18:00 ,,	—	7.00
24:00 ,,	—	9.00
30:00 ,,	5.15	9.00
36:00 ,,	9.15	—

laboration with the Bioassay Research Unit. The Galleria bioassay was utilized. J.H. titres of larvae at various ages after moulting into the last instar were checked. Results obtained to date are presented in Table 3. These results are not complete as yet but are presented here to give an insight into the phenomenon of phase-variation. There seems to be a general increase in the J.H. titres gradually after an initial low, in both the categories. The titres in "Solitary" larvae were generally a little higher than those of the "gregarious" ones.

Effects of topical application of J.H. on 6th instar larvae
In an effort to check if application of J.H. to the larvae has any effect on their development, experiments were carried out using juvenile Hormone I and III. Larvae moulting into their 6th instar were chosen for testing. Treatments were as follows: 1. 10µl applied each day starting day one, 2. 10µl applied only on day one, 3. 5µl applied each day and 4. 5µl applied only on day one. Application of the solvent (Acetone) each day acted as control. Ten larvae per treatment were used. Applications of J.H I produced slightly longer durations during the 6th instar, prepupal and pupal stages when compared to the control. The durations in the daily applications were also a little longer in relation to first day applications. Applications of J.H. III did not produce any noticeable differences in the durations. The pre-pupae shrunk and shrivelleged and considerable mortality was noted, suggesting probable morphogenetic effects.

Studies on *Spodoptera exempta* in Outbreak areas

Jacob Yarro
During the armyworm outbreak season of 1979 a number of outbreak areas were visited. Larval and pupal densities were determined in stands dominated by certain species of grasses. The densities were determined by taking counts in randomly chosen one square meter quadrats. The number of quadrats varied from three to six for each stand. In the determination of pupal densities the boundary of the quadrat was marked and the pupae were carefully dug out and collected for counting. The mean densities of larvae or pupae for each grass species were based on the number of quadrats sampled for that particular stand.

The pupae collected from the field were taken to the laboratory and kept indoors until emergence. Male and female weights were taken within twelve hours of emergence and after meconium had been voided. Comparisons were made between emergence weights of moths from the various grasses in each locality. In addition emergence weights of moths collected from stands of *C. dactylon* in three localities were compared.

In all the four localities the highest larval and pupal densities were in areas where the predominant host plant was *C. dactylon* (Tables 4 and 5). The proportion of total population of larvae becomes higher on *C. dactylon* in habitats which are less favourable for growth of grass (Athi River and Kajiado). The higher rainfall at Athi River leads to better growth of grass of all species whereas lower rainfall at Kajiado results in poor growth of grasses. This means that even if the initial densities were similar, the differences in food quality would lead to differential survival and therefore different densities. In areas with more favourable conditions for growth of grasses the larvae are more evenly distributed on various grasses whereas in drier areas the larvae are more concentrated on *C. dactylon*.

Prior to pupation the larvae search for adequate pupation sites. These may be in the area where they fed but may also extend to other sites. Although the pupal densities are higher in areas with *C. dactylon*, the pupal density at Taveta was found to be higher in an almost bare patch of land than in areas with some of the species of grasses.

The emergence weights of moths from various grass species at both Kajiado and Taveta did not vary significantly from each other. These were, however, significantly different from each other at Athi River where females were significantly larger than males (Table 6). The optimum conditions for grass growth at Athi River probably leads to larger moth size than observed in drier areas.

This is perhaps better demonstrated by the populations obtained from *C. dactylon* in three localities (Table 6). The largest moths were obtained from Athi River with the smallest ones from Kajiado. The emergence weights of moths from Taveta lay in between. These differences are significant. The results show that different grass species under similar environmental conditions gave different effects on the survival and general biology of the insect. Furthermore, the same host plant species under varying environmental conditions affect the survival and biology of the insect differently.

Table 4. Larval densities of *S. exempta* in outbreak areas

Locality	Host plan	Mean/m² ± S.E	Percentage
	Cynodon dactylon	135.00±8.66	35.13
	Themida triandra	93.00±9.07	24.20
Athi River	*Pennisetum mezianum*	67.33±10.74	17.52
	Panicum maximum	54.33±8.09	14.13
	Aristida keniensis	34.67±3.18	9.02
	C. dactylon	49.75±6.25	57.18
	Digitaria spp.	16.00±3.34	18.39
Ngong Race-Course	*T. triandra*	10.75±2.90	12.36
	P. maximum	10.50±1.50	12.07
	C. dactylon	154.50±5.42	41.34
	P. mezianum	79.25±3.94	21.20
Magadi	*P. maximum*	66.25±8.90	17.73
	Cenchrus ciliaris	61.00±3.72	16.32
	A. keniensis	12.75±1.93	3.41
	C. dactylon	10.67±2.28	71.51
	T. triandra	2.00±0.91	13.41
Kajiado	*P. maximum*	1.00±0.58	6.70
	Digitaria spp.	0.75±0.75	5.03
	A. keniensis	0.50±0.20	3.35

Note: For each grass species there were four quadrats except Kajiado where six quadrats were sampled.

Table 5. Pupal densities in various grasses in three localities

Locality	Host Plant	Mean/m^2±S.E	Percentage
Taveta	*C. dactylon*	108.33±9.28	45.45
	Between a ploughed field and a stand of *C. dactylon and P. meziauum*	56.67±11.56	23.78
	P. maximum	49.33±4.81	20.07
	P. mezianum	24.00±2.65	10.07
Athi River	*C. dactylon*	121.67±3.68	53.68
	T. triandra	46.00±4.58	20.29
	A. keniensis	34.67±15.24	15.29
	P. maximum	23.00±1.00	10.15
	P. mezianum	1.33±0.67	0.59
Kajiado	*C. dactylon*	132.67±4.73	82.23
	T. triandra and P. mezianum	28.67±6.35	17.77

Note: For each grass species there were three quadrats of one square metre each.

Table 6. Emergence weights of *S. exempta* moths from three localities

Locality	Host plant	Male weight±S.E (mg)	Female weight±S.E (mg)
Taveta	*C. dactylon*	67.86±2.36 (28)	74.68±2.47 (29)
	Between a ploughed field and a stand of *C. dactylon* and *P. mezianum*	61.68±4.29 (8)	65.81±3.73 (14)
	P. maximum	63.17±2.38 (28)	75.07±7.19 (4)
Athi River	*C. dactylon*	82.88±6.14 (20)	98.75±8.88 (7)
	A. keniensis	75.63±2.37 (29)	80.07±2.54 (38)
	T. triandra	77.87±6.15 (10)	91.57±5.28 (11)
	P. maximum	71.56±3.65 (19)	72.76±2.62 (29)
Kajiado	*C. dactylon*	62.62±1.81 (59)	65.74±1.61 (57)
	P. mezianum and T. triandra	44.71±4.22 (17)	47.85±8.95 (5)

Note: The number of moths weighed are in parenthesis.

Physiological age of *Spodoptera exempta:* morphology of the female reproductive system

B. Otindo

Considerable work has been done on the African armyworm moth *S. exempta* but there is little information on its population age structure. In order to undertake studies on age determination methods, a supply of basic information on the reproductive system was necessary. The female reproductive system is quite conspicuous and therefore initial investigation was carried on it.

Morphology of Ovaries

The mature ovaries and the bursa copulatrix are the most conspicuous parts of the female internal reproductive system. In a female of oviposition age of 4 days, the paired ovaries extend into the 3rd abdominal segment. Each ovary consists of four polytrophic ovarioles, and has extensive loops. The loops are more pronounced at the distal ends where the ovarioles adhere very closely to each other that they appear as a single strand (Figure 4).

Maturation of oocytes can be distinguished morphologically without the use of stain techniques. However, exact maturation rate can be determined precisely by staining with such a desirable compound as methylene blue which differentiates the chorion from the ooplasm. Immature eggs are characterised by the presence of a cap of nurse cells (Figure 5). The nurse cells degenerate and disappear completely as oocytes mature fully.

Bursa copulatrix

The external opening of the bursa copulatrix is located in the intersegmental membrane between the 7th and 8th abdominal sternites. The opening is labelled as the ostium bursae (Figure 6) and leads into the corpus bursae.

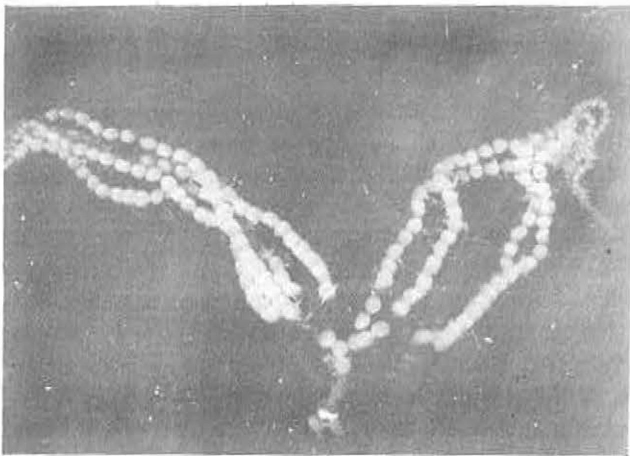


Figure 4. *Spodoptera exempta* ovary of a female of oviposition age.

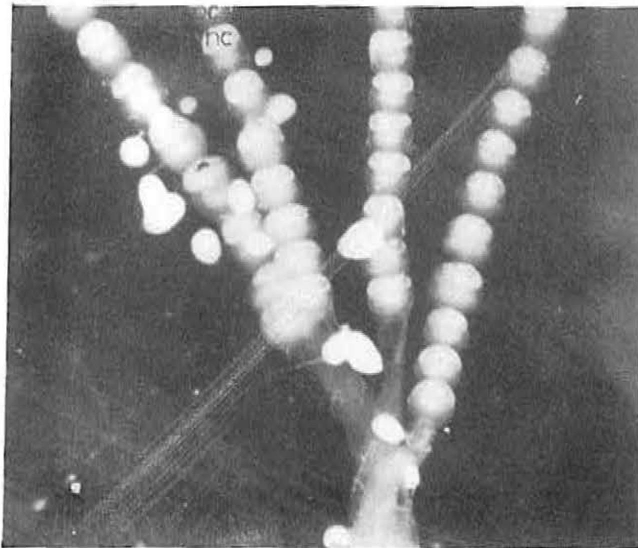


Figure 5. *Spodoptera exempta* immature ovarioles, nc cap of nurse cells, OC oocyte.

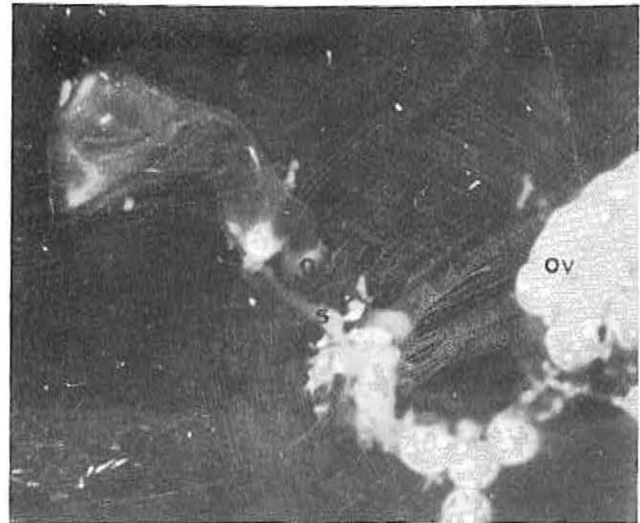


Figure 6. *Spodoptera exempta* bursa corpulatrix. O ostium bursae, Ov ovary, S. Seminal duct.

To determine the mating history of moths, the muscles of the corpus bursae are dissected out. Laboratory studies have shown that one mating only can take place in a night (Khasimuddin, 1978), thus confirmation of mating is useful for approximate ageing.

Hormonal control of larval colouration associated with phase variation in the armyworm, *Spodoptera exempta*

S. Yagi

It is well known that *S. exempta* larvae have phase variation exhibited as colouration and behavioural changes in response to population density. "Gregarious" larvae always show darker colouration, while "solitary" ones are light in colour. The hormonal control of larval colouration is being studied in relation to phase variation in this armyworm. Some preliminary results are reported below. Observation of colouration in the larvae was performed on the integuments attached on filter paper (Figure 7).

Timing of 5th larval ecdysis

Head capsule of the 6th instar larva becomes visible under the integument of the capsule of the 5th instar larva before the last larval ecdysis. The time elapsed from this stage to ecdysis was observed. Most of the gregarious larvae ecdysed to the 6th instar 21($\pm$2.5) hr after the new head capsule became visible.

Ligation of gregarious larvae

When the 5th instar gregarious larvae 21($\pm$2.5) hr before the last larval ecdysis were ligated behind the thorax, almost all larvae ecdysed to the 6th instar within one day. After ecdysis the old cuticle was removed and the colouration between anterior and posterior part of the larva was checked. In most cases, the colouration

of the anterior part was darker than that of the posterior. However, there was no difference in the colouration of these regions if the larvae were ligated 3–5 hr before ecdysis.

The results suggest that some endocrine organ(s) located in the anterior part may produce (a) hormone(s) to cause dark colouration of the gregarious larvae.

Extirpation of neuroendocrine organs from gregarious larvae

The suboesophageal ganglion (SG) was removed from the 5th instar gregarious larvae 21($\pm$2.5) hr before ecdysis also using extirpation of the metathoracic ganglion (MG) and sham operation as controls. The dark colouration of the larvae was reduced by the removal of SG (Figure 7c) but did not change in control larvae (Figure 7d).

The results indicate that SG may play a role in induction of dark colouration of the gregarious larvae. Extirpation of other organs such as brain, corpus cardiacum-corpus allatum complexes and other ganglia is in progress.

Effects of MRCH on solitary larvae

MRCH (melanization and reddish colouration hormone) obtained from the silkmoth, *Bombyx mori* was injected to 5th instar solitary larvae about 21($\pm$2.5) hr before ecdysis. After ecdysis the dark colouration of the larvae greatly increased and was comparable to that of the gregarious ones (Figure 7E). These are preliminary results, but it is suggested that MRCH may cause dark colouration of *S. exempta* larvae as has been reported for *Leucania separata*. Further studies of the role of juvenile hormones or ecdysteroids in colouration of *S. exempta* larvae are continuing.

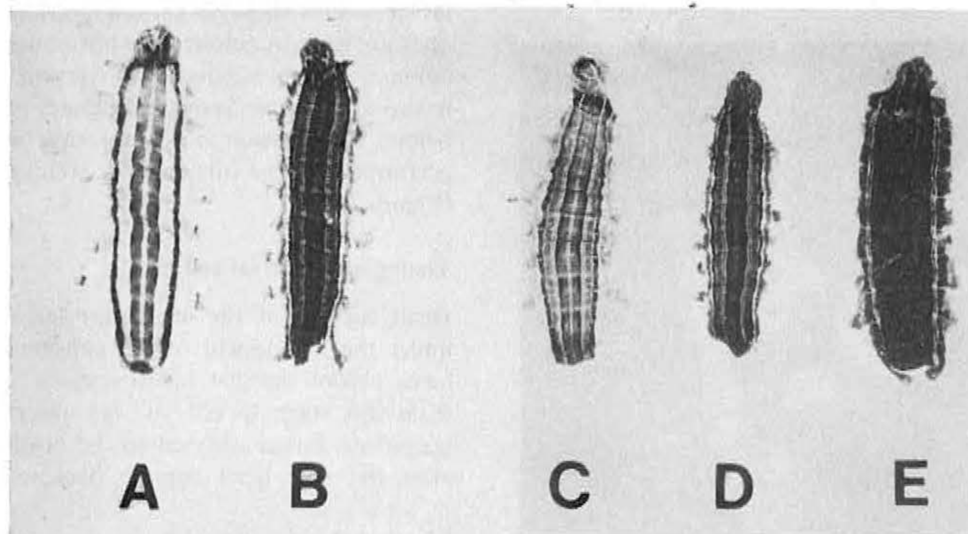


Figure 7. Typical examples of 1-day-old 6th instar larvae of *S. exempta*;

A. Solitary larva
B. gregarious larva
C. gregarious larva extirpation of SG
D. gregarious larva after extirpation of MG
E. solitary larva after injectiou of MRCH,

BASES OF PLANT RESISTANCE TO INSECT ATTACK RESEARCH PROGRAMME

Acting Programme Leader
Dr. Z. T. Dabrowski (1979)

Research Staff

Mr. E. O. Nyangiri (1979) Senior Technician
Dr. R. S. Ochieng' (1977) Postdoctoral Research Fellow
Mr. P. O. Odinga (1978) Junior Technician
Mr. S. H. Okech (1977) Technician, IRRI, Philippines
Mr. E. O. Omolo (1978) Agronomist
Mr. F. O. Onyango (1979) Technician
Miss A. Ragot (1978) Technical Assistant
Dr. R. C. Saxena (1977) Research Scientist, IRRI Philippines

Collaborators

Dr. A. Raina, Sorghum Shootfly Research Project
Dr. W. Otieno, Insect Pathology
Mrs. N. Y. Patel, Salivary Gland Physiology
Dr. D. Whitehead, Chemistry and Biochemistry
Dr. T. Gebreyesus, Bioassay
Dr. E. D. Kokwaro, Histology and Fine Structure
Mr. J. Ongudha, Insect and Animal Breeding

Introduction

Z. T. Dabrowski

The use of insecticides is the most common and conventional method of plant protection against insect pests. However, the development of resistance in insects to insecticides, the increase in the cost of inputs during the last ten years together with public awareness of problems relating to the use of insecticides have caused increased interest in developing alternative methods of plant protection.

Among the various alternatives to insecticides, the use of insect resistant plants, in combination with good cultural practices is the most effective, convenient, economical and environmentally acceptable method of insect control. In addition, it is a method that is completely compatible with both chemical and biological measures.

The use of resistant cultivars as a method of crop protection has gained acceptance in tropical countries. Host plant resistance is now considered to be one of the primary lines of defence in all pest management programmes for small farmers. Introduction of new resistant cultivar releases farmers from worrying about technological aspects such as timing of application, dosage of a chemical or biological agent and there is no direct cost to growers. The latter two considerations make the use of resistant plants important to both developed and developing countries, and they should thus form an integrated part of a pest management programme.

It must be stressed, however, that while plant resistance to insects is a highly promising strategy of pest control, it requires sustained long range work and a joint action of research by entomologists, agronomists, plant breeders and geneticists, plant and insect physiologists and chemists.

Detection of mechanisms and genetic factors involved in plant resistance usually takes 3–4 years and the release of a new resistant cultivar requires about 6–10 years.

Host plant/insect relationships and studies on plant resistance in the ICIPE in 1979

Z. T. Dabrowski

Plant resistance to insects is defined by Painter (1951) as the consequence of heritable plant qualities that results in the plant being relatively less damaged than a susceptible plant without these qualities. The final effect of exhibition of plant resistance is expressed in reduction of pest reproduction and development as determined from successive relationships between plant and insect populations throughout their separate mechanisms of mutual adaptation. Both plant and pest populations are heterogeneous and consist of specimens with various degrees of adaptations. Thus the level of natural adaptation may fluctuate forming favourable conditions for either plant populations increasing their insect resistance level or for increasing mass reproduction in the pest population. The degree of heterogeneity in insect and plant populations is a most important aspect in the study of plant resistance to pests, since mainly the elite section of the insect population takes part in the process of its reproduction while the part of the plant population possessing the highest degree of resistance, accounts for species preservation during the process of natural selection.

A project on plant resistance to insects was started in 1978, the research work being concentrated in the Mbita Point Field Station and in the International

Rice Research Institute (IRRI) in the Philippines. Extensive studies were initiated in September, 1979 with funds provided by the USAID.

The research is conducted on four levels:

(1) Field screening of recent and old cultivars and lines. originating from international plant breeding institutions (IRRI, ICRISAT, IITA, CIMMYT) and from Kenya. The screening requires estimation of reliable and efficient screening techniques. Where artificial infestation is necessary, procedures for mass rearing of the insect species tested are elaborated. Screenings are conducted at the Mbita Point Field Station.

(2) Studies on mechanisms of plant resistance include all aspects of behavioural and physiological relationships between insect pest and crop plant tested. Insect resistance in crop plants could be expressed in several ways. In our research programme we distinguish these mechanisms of resistance:

(i) NON-ACCEPTANCE — Resistance that adversely affects the behaviour of an insect in search for food, oviposition and/or shelter in a situation when the level of infestation of a cultivar is compared on adjacent plots with other more sensitive plants. This resistance must be confirmed with the cultivar being grown on a large scale. Plant resistance to oviposition may be conditioned by characteristics acting either by failing to provide the appropriate positive stimuli (attractants and arrestants) for one or more of the behavioural components, or by providing negative stimuli (repellents) that inhibit behavioural release. Tactile proprioceptive, chemotactic and visual factors may play a role in site selection and subsequent egg deposition.

Feeding involves a sequence of the following stereotyped behavioural components: (a) host plant finding: (b) host recognition (c) host acceptance. Each of these innate behavioural components manifests itself only in response to a proper combination of plant properties and instrinsic response threshold levels. The insect may be actively repelled by the plant, moving away from it, without even coming into contact with it, or having made contact further feeding activity such as biting may be suppressed; or having bitten the leaf, the insect may be deterred from feeding. The chemicals involved in inhibiting feeding behaviour at these points are summarised by Beck as repellents, suppressants or deterrents.

Our investigations on mechanisms involved in oviposition behaviour of adult insects and larval feeding behaviour were initiated at the ICIPE not only for the purpose of studies on plant resistance, but also to find the best techniques for insect mass rearing. Without detailed information on plant properties affecting the insect in its search, recognition and acceptance of a plant as food, it would be difficult to choose the optimal artificial surface for oviposition or to compose artificial diets for larvae.

At present, our work on this topic is concentrated on describing the physical and chemical characteristics of cowpea plants influencing female behaviour and larval feeding behaviour of the cowpea pod borer, *Maruca testulalis.*

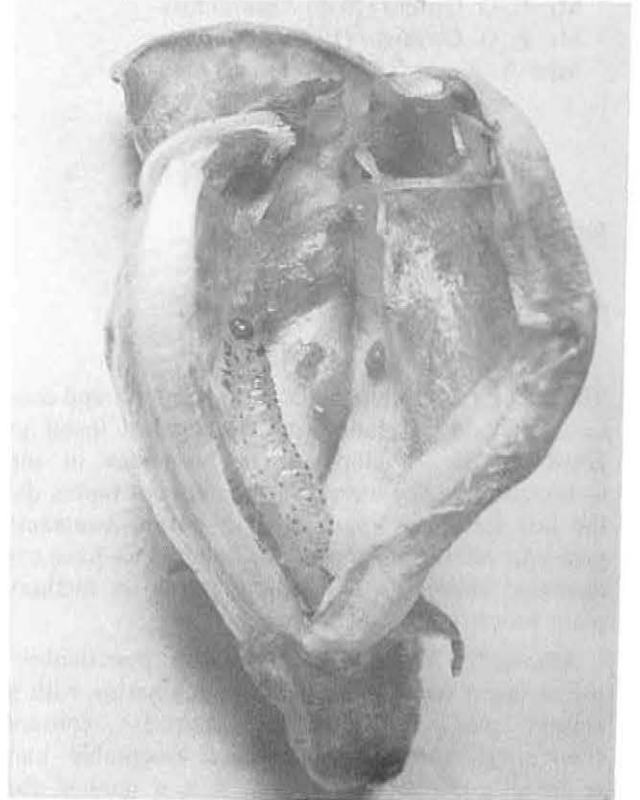


Figure 1. Larva of the cowpea pod borer, *Maruca testulalis* feeding in the cowpea flower.

The active compounds would be classified as attractants, arrestants, feeding incitants or feeding stimulants.

(ii) ANTIBIOSIS: This comprises defensive mechanism of plants against their pests through adverse influence by means of chemical or morphological factors on growth, survival or reproduction of the insects feeding on the resistant plants.

Antibiosis as the mechanism of sorghum resistance to *A. soccata* larvae was mentioned by Ponnaiya (1951) and Blum (1967, 1968), who associated the sorghum resistance with

some anatomical characteristics of resistant cultivars. They found high correlation between plant resistance and the density of silica bodies in the epidermis of the base of the leaf sheaths and lignification and thickness of the walls of cells enclosing the vascular bundles within the central whorl of young leaves.

They did not, however, prove if the anatomical resistance of sorghum seedlings to larval penetration may be physical because it restrains larval movement in plants, or because of the impossibility of larvae to feed and develop on tissues of resistant plants. The variation in food intake and utilization by insects on various crop plant cultivars is one of the resistant mechanisms classified as nutritional antibiosis.

The sorghum shootfly larvae after hatching on the leaf surface move down between the leaf sheath of basal portion of the leaf. On reaching the base, it cuts through the ventral surface of the other under-developed leaf sheath and cuts horizontally the central core of plant. The growing apex is separated completely from the basal portion and larvae feed on the decaying central shoot.

The assumption was made that for completely understanding the feeding behaviour and nutrition of *A. soccata* larvae on various cultivars of sorghum, it is necessary to investigate the relationship between plant chemicals occurring in resistant sorghum plants and digestive enzymes of larvae. Such studies are now being initiated by Dabrowski, Patel and Whitehead at the ICIPE. However, at least an introduction to the anatomical organization of the alimentary canal of larvae is needed to understand the digestion and absorption of food taken from resistant and susceptible sorghum lines.

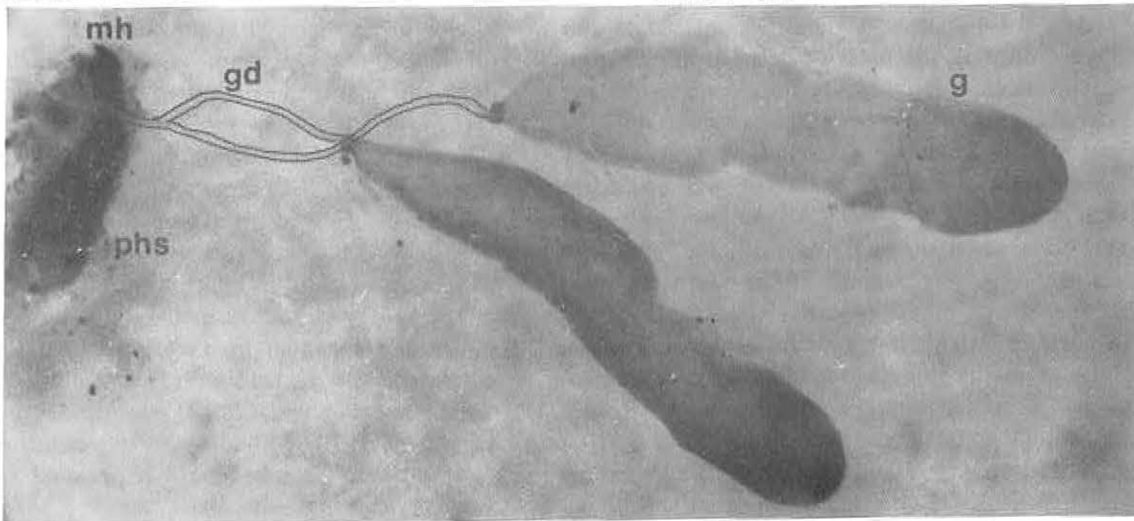


Figure 2. Salivary glands of the third larval instar of *Atherigona soccata;* mouth hook (mh), pharyngeal sclerite (phs), gland ducts (gd), glands (g).

We started work on the location and morphology of salivary glands of the third larval instar. The pair of glands lying below the mesenteron of alimentary canal. Their average length is 1.68 (range 1.44—1.96) mm. and average width is 0.37 (range 0.26–0.44) mm. They are connected by two ducts running anteriorly to a single unit and open into the preoral cavity (Fig. 2).

Antibiosis acts by interfering with physiological processes underlying insect growth, metamorphosis, and reproduction. Such physiological effects may be caused by metabolic inhibitors (allelochemicals) in the plant tissues, or by the plant failing to provide specific nutrients or nutrient balances required by the insects.

Although allelochemicals themselves are by definition non-nutrient, they can interact with essential nutrients. Many of the deleterious metabolic and chronic effects of plant allelochemicals may actually be due to such interaction. Some allelochemicals apparently interfere with nutrients by blocking their availability. Growth inhibition may be due to reduced assimilation, reduced efficiency of conversion of assimilated food, or a combination of both.

(iii) TOLERANCE: This is the ability of plants to withstand damage caused by an insect population or recover from amount of damage approximately equal to that damaging a susceptible host. This type of resistance might therefore be termed the recovery resistance (Painter 1951).

Research on the mechanisms of plant resistance is conducted in co-operation with other units

of the ICIPE: chemistry; microbiology; physiology, histology and electrophysiology.

3. Complementary genetic studies on the inheritance of resistance: The breeding programme for maize resistance will start with the determination of genetic factors conditioning resistance. The resistance of individual plants in segregating offsprings will be evaluated under Mbita Point conditions during the summer of 1980 to identify the genes responsible for resistance:
4. Genetic and physiological mechanisms responsible for development of new insect biotypes developing on resistant lines and cultivars:
   These studies were initiated by Dr. Saxena on biotypes of the rice brown planthopper, *Nilaparavata lugens*, developing on certain resistant rice lines. Since at the moment this pest is of great importance in South East Asia and West Asia but not in Africa, these studies are conduted in the IRRI, Philippines.

The search for insect resistance in crop plants can be conducted only on the basis of detailed information on the bionomics, ecology, injury levels and behaviour of the insect species under different environmental conditions. Information on the ecology and biology of the insect species studied in our project originates from data collected previously by entomologists at the ICIPE or by scientists in Kenya and other countries in Africa or Asia. When such information was not available for Kenya conditions, the scientists involved in our project had first to collect fundamental information on the biology and ecology of the insect species studied. Dr. Odebiyi initiated such investigations on *Maruca testulalis* biology under the Mbita Point Field Station conditions. His report is included in the Crop Borers Programme because of its relevance to the biology and ecology of other borers.

The project started under difficult conditions caused by the temporary lack of sufficient laboratory and insectary facilities at the Mbita Point Field Station and at Nairobi and by vacancies in scientific and technical staff. Because of these vacancies, our scientists could not concentrate on studies on the relationship between one pest species and one crop plant.

Significant progress was achieved by Dr. Saxena on rice resistance to the rice brown planthopper, *Nilaparvata lugens* in the IRRI in the Philippines and by us by experiments on the relationships between *Chilo partellus*, *Atherigona soccata* and *Maruca testulalis* and their host plants. The construction of four screen houses for our projects in the Mbita Point Field Station allowed us to start an extensive research programme on the methodology of screening of maize and sorghum to stem borers under artificial infestation in September. The envisaged building of a greenhouse in Nairobi will create conditions to start on co-operative research with other units of the ICIPE on mechanisms of plant resistance to insects.

PRELIMINARY OBSERVATIONS ON MAIZE AND SORGHUM INFESTATION BY VARIOUS SPECIES OF STEM BORERS UNDER THE MBITA POINT FIELD CONDITIONS

R. S. Ochieng

A new project started at the Mbita Point Field Station on maize and sorghum resistance to insect species damaging plant stems required information on:

(i) species composition of stem borers damaging sorghum and maize under West Kenya conditions;
(ii) biology of the borers;
(iii) distribution of insects on the plant infested in the field
(iv) effect of plant infestation on yield.

The aim was to estimate the infestation levels of maize and sorghum by the spotted stalk borer (*Chilo partellus*), the maize borer (*Busseola fusca*) and the pink stalk borer (*Sesamia calamistis*) during the dry season of 1979. It had been noticed that the above named borers attacked both maize and sorghum with varying degrees of intensity.

Sampling was carried out on a fortnightly basis beginning with the reproductive stage—Stage 7 (tussting) of plants in the second week of October. A random sample was taken from the 30 metre square maize plot and from the 30 metre square sorghum plot. Fifty plants were sampled each time. Three samples were taken.

Table 1 shows the distribution of these borers in both maize and sorghum. In maize there was an equal distribution of *Chilo* and *Busseola*. On the other hand *Sesamia* distribution was always very low.

Table 1. Species composition of the population of three major stem borers damaging maize and sorghum in the dry season of 1979

Sample	MAIZE			SORGHUM		
	Chilo	Busseola	Sesamia	Chilo	Busseola	Sesamia
1	107	108	2	244	0	1
2	157	172	8	113	35	25
3	80	69	4	90	12	23
Total	344	349	14	447	47	49
Species composition	48.8%	49.3%	1.9%	82.4%	8.6%	9.0%

In sorghum the major borer was *Chilo*. *Busseola* and *Sesamia* were of insignificant consequence. These are only preliminary observations and further work on the ecology of these stem borers is planned for the next long rain period.

The programme of maize breeding for resistance in Western Kenya should be focused on these two pest species, and the analysis of varietal differences in plant damage level should be done separately for each species.

SCREENING MAIZE AND SORGHUM CULTIVARS FOR RESISTANCE TO STEM-BORERS COMPLEX

E. O. Omolo

The purpose of this study was to select, screen and develop maize and sorghum genotypes with the following characteristics; more efficiency in grain production, medium growing period (4–5 months), shorter stature, and higher resistance to diseases and to stem borers.

One hundred different maize cultivars, late, medium and early maturing cultivars as well as coastal and high altitude maize, drawn from five region maize programmes in Kenya were observed. One hundred sorghum varieties were also screened. Among them were local varieties from all parts of Kenya, material from the former EAAFRO-Serere, Uganda; Ukiruguru—Tanzania, Alemaya—Ethiopia and ICRISAT in India.

Due to the large numbers and the limited amount of seed, only one replication was planted mainly to increase and renew the seed and at the same time carry out preliminary observations. Maize was planted in plots of two rows of fifteen plants each. The spacings were 75cm. between the rows and 30cm. between plants within the row. In cases where seeds were limited only one row was planted. Similarly, sorghum lines were planted in plots of two rows of ten plants per row, with spacing of 60cm. between rows and 15cm. between plants in a row.

The cultivars were described as adapted (+), unadapted (—) and in between (±) depending on their suitability to the area in question. Mbita Point Field Station, in Western Kenya falls into the medium maturing maize area, i.e. 4–5 months and therefore both late and early maturing varieties covering 7–8 and 3–4 months respectively were described as unadapted, however, 3–4 months maturing ones would pass during marginal seasons and that was the reason for describing them as (±).

Table 2. Infestation of relatively resistant and sensitive maize cultivars by stem borers

Cultivars	Source	Variety Adaptation	No. of Plants damaged/total	% Infestation
1. Nyamula I	Lambwe	+	2/24	8.0
2. Nyamula II	,,	+	2/25	8.0
3. Inbred D	Kitale	+	2/20	10.0
4. Kitale Sym. II	,,	±	1/10	10.0
5. H 611	,,	—	2/21	10.0
6. Katumani SR 52	Machakos	+	2/20	10.0
7. KCE—15	C. America	±	1/8	12.5
8. SR 52	Zambia	±	3/21	14.2
9. Nyamula III	Lambwe	+	4/24	16.0
10. Radier	,,	+	5/27	18.0
$\bar{x}$	Mean of ten			11.67
26. Wang'e dongo	Lambwe	+	18/21	86.0
27. Inbred A	Kitale	±	23/25	92.0
28. KCB—58	Machakos	±	7/8	90.0
29. KCB—30	,,	±	4/5	80.0
30. KCE. Co.	C. America	—	4/5	80.0
$\bar{x}$	Mean of five			85.6
$\bar{x}$	Over all Mean			36.85

Table 3. Infestation of relatively resistant and sensitive sorghum cultivars by stem borers

Cultivars	Source	Variety Adaptation	No. of Plants Damage/total	% Infestation
1. Sorghum—117	Machakos	±	2/21	10.0
2. 5D × 135/13/1/3/1	EAFRO	+	5/15	30.0
3. IS 8315 × 407	ICRISAT	±	3/8	37.5
4. IRA—761	Ethiopia	—	3/7	42.8
5. IRA—708	,,	—	2/6	35.0
6. Kafinama x SB 65	EAAFRO	+	8/20	40.0
7. IAR—1742	Ethiopia	—	6/15	45.0
8. Ex-Mombasa	Mombasa	±	6/15	45.0
9. IAR—1881	Ethiopia	—	4/9	45.0
10. Ex-Mukaa	Machakos	+	4/9	45.0
x̄	Mean of ten			37.5
25. Andiwo Rabuor	Lambwe	+	4/5	80.0
26. Ex-Makueni	Machakos	±	12/14	85.0
27. Sorghum—15	Machakos	±	6/7	85.0
28. Andiwo Rachar	Lambwe	+	7/8	87.0
29. Nyakandete	Kisii	—	9/10	90.0
30. Sorghum—1291	Lanet	+	13/14	92.0
x̄	Mean of six			86.5
x̄	Over all Mean			77.5

Adaptation = + Adapted variety.
— Not adapted variety.
± In between.

Maize	Resistant—Inbred D*
	Kitale II?
	Nyamula
	Moderate—Inbred G*
	KCB
	Embu
	Sensitive— Inbred A*
	Rachich (Purple)
	KCE
Sorghum	Resistant—Sorghum 117*?
	Sorghum 5D
	Moderate—Sorghum 65*
	Sorghum 708
	Ex Mukoa
	Sensitive—Sorghum 1291*
	Sorghum 115
	Ex Makueni I

*—Cultivars selected for future screening purposes.

?—The populations from which lines would be derived.

The results are provisional and recommendations based on them should be treated with caution, however, they may serve as baseline information for further screening and for methodology studies on bases of resistance to insect attack.

ESTIMATION OF METHODOLOGY OF MAIZE SCREENING TO *CHILO PARTELLUS* INFESTATION

Z. T. Dabrowski and E. Omolo

To distinguish between escape of plants from insect attack and genuine resistance under field conditions, the maize lines tested must be exposed to high and uniform pest infestation.

Future progress in breeding resistant cultivars may be considerably retarded by including lines which have been wrongly classified as resistant while in fact they are insect-escaping. The escape of plants may be eliminated by the application of homogeneous artificial infestations or by the increase of wild insect population under field conditions. To obtain sufficiently large quantities of insects of the appropriate stage at a given moment of plant development, specific artificial rearing techniques are necessary.

Estimation of methodology of maize screening to *Chilo partellus* infestation for our future mass screening and breeding programme has required some preliminary field and laboratory experiments be conducted on:

A various techniques used for artificial manual infestation

B Storage of *Chilo partellus* eggs and/or larvae under low temperatures for screening purposes.

(A) The aim of our experiments set up in October 1979 in the Mbita Point Field Station was to compare various methods used for artificially infesting maize plants and for rapidly evaluating their damage level. The following variable factors were taken under consideration after consultation with Dr. Marcel Hudon, Assistant Director in charge of the maize programme, St. Jean Research Station, Quebec, Canada.

(i) artificial infestation vs natural field infestation
(ii) eggs vs larvae used in artificial infestation
(iii) quantity of eggs on larvae per plant
(iv) time of artificial infestation (morning vs afternoon)
(v) place of plant infestation with masses of eggs (fully developed leaves vs young leaves vs plant whorls);
(vi) growth stage of maize (from No. 3 to No. 6) to be suitable for infestation;
(vii) growth stage of maize adequate for evaluation of leaf and stem damage;
(viii) estimation of plant damage on the base of leaf damage (1–9 class leaf-feeding rating scale) vs the extent of plant damage (another) (1–10 scale);
(ix) the criteria for evaluation of stem damage (number of cavities vs the number of cm of tunnelling vs the ratio of length of tunnels/plant height).
(x) damage index vs decrease of yield of three maize cultivars previously described as resistant, moderate resistant and sensitive to *Chilo partellus* infestation under the Mbita Point conditions.

Starting November 26, maize plants of stage four were once or twice manual infested with egg masses (about 50 eggs) by fasting the paper discs with eggs onto the bottom side of the last fully developed leaf or by dropping the eggs deep into the whorl. To eliminate eggs evaporation the infestations are performed in the afternoon, between 4–8 p.m. Only egg masses at the black head stage (about half day before hatching) are deposited on plants to prevent their destruction by ants or other predators.

Leaves of infested plants are classified every second week into 1–9 classes depending on the number and size of holes Fig. 3. This is for the antibiosis of plants against the borers. After one month from infestation more ratings are made on (over-all damage) a scale 1–10 for total plant damage to evaluate the tolerance of the plant until harvest. Statistical analysis of periodically collected data should show the optimal period to evaluate leaf and plant damage.

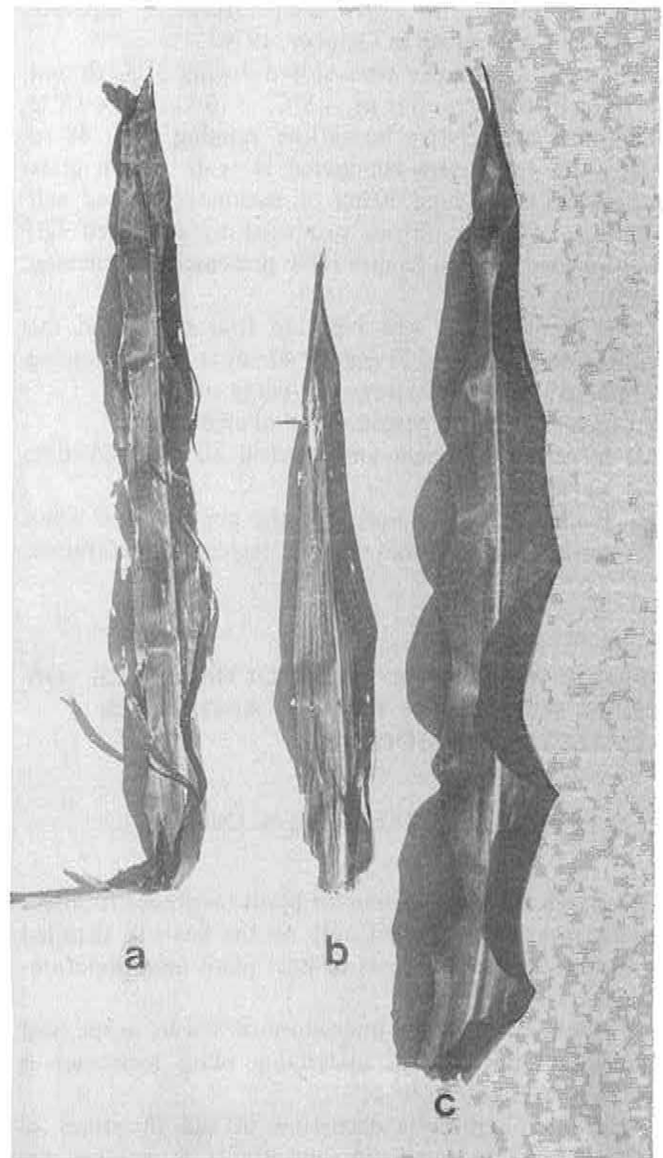


Figure 3. Maize leaves: seriously damaged (a), moderately damaged (b) and undamaged (c) by stem borer larvae.

The third evaluation is yield of plants, the number and length of tunnels evaluated during harvest.

(B) In the mass screening of plant to insect attack under artificial infestation, the insect production has to be synchronized with the development of plants tested. In our screening programme of maize and sorghum cultivars and lines starting in the long rain period of 1980 we will need thousands of *Chilo* eggs or larvae. Under the present restricted accommodation conditions at the Mbita Point Field Station an enlargement of insect production on artificial diets to cover all the needs of the plant screening programme is not possible. It was therefore decided to rear the insects in the Insect and Animal Breeding Unit in Nairobi and send the eggs or pupae to the Mbita Point Field Station.

The shipment and the screening procedure sometimes requires that eggs or pupae be stored for some days in Nairobi or later in the Mbita Field Station. To estimate the optimal condition for storage of *Chilo* eggs and pupae in lower temperatures, a separate experiment was set up in October, 1979.

The eggs and pupae were stored during 3, 5, 10 and 15 days in temperatures of +5°C, +10°C and +15°C and under six relative humidities ranging from 45 to 100%. All tests were conducted in wide mouth glass desiccators containing 300ml of various saturated salt solutions. The humidities produced by saturated salt solutions were taken from a table presented by Peterson (1970).

One combination was repeated four times and the replication consists of 25 eggs or 40 pupae. The following parameters of *Chilo* biology are being observed.

(i) percentage and period of larval emergence;
(ii) larval development and survival on artificial diet;
(iii) pupae survival;
(iv) female fecundity (only for the combination when pupae were stored under lower temperatures).

EFFECT OF SOME ALLELOCHEMICALS ON *CHILO PARTELLUS* LARVAE AND THEIR SYMBIOTIC MICROFLORA

Z. T. Dabrowski, W. Otieno and R. Ochieng

The search for mechanisms for plant resistance to insect attack may be conducted only on the basis of detailed information on all aspects of host plant/insect relationships.

The role of intestinal microflora of insects in the host plant/pest relationships underlying plant resistance is not very well known.

The microorganisms occurring in the intestines of healthy insects may be important as synthesizers of growth-promoting substances or may promote immunity to pathogens by inhibition of pathogenic bacteria The microflora depends quantitatively and qualitatively on the physiological state of food plants and degree of bacteriophage activity of secondary metabolic substances (allelochemicals) contained in plant tissues. A concentration of allelochemicals in plants differs in various crop varieties and are identified in most cases as the plant compounds responsible for antibiosis, which adversely affects larval survival and development on resistant cultivars.

Most of the previous work on antibiosis had concentrated on the study of the direct effect of phytotoxins upon insect development and overlooked the possible indirect influence of the allelochemicals on insect physiology and nutrition. Therefore some allelochemicals affecting the microflora of larvae may cause nutritional deficiences instead of acting as direct larval phytotoxins.

Studies on the intestinal microflora of the *Chilo partellus* larvae collected from maize and sorghum growing under the Mbita Point Field Station were started in September 1979. The larvae were sent in pieces of sorghum or maize stems to the Mombasa Station for identification of species composition of their microflora.

The larvae were disinfected by washing them in a solution of 0.1% mercuric chloride in 70 per cent alcohol. The insects were held in the disinfectant for 15 minutes. At the end of the disinfecting time the specimens were thoroughly washed in sterile water or saline to remove all traces of the disinfectant. This usually required six to seven complete changes of the washing fluid.

After the larvae had been satisfactorily washed, they were placed in a sterile petri dish and dissected to remove the gut. The gut was triturated with an abrasive using sterile saline as a diluting medium.

Aliquots (5 ml.) of the solution containing gut flora were transferred onto solid Nutrient Agar Medium placed in an incubator at 38°C.

Identification of the bacteria isolated were carried out through a study of the organisms' morphologic, physiologic, and cultural characteristics. Morphologic characteristics were obtained by using Giemsa's and Gram's stains. The physiological and cultural characteristics were studied by the use of differential media-gelatin and carbohydrate. After this had been accomplished, an accepted standard of classification was consulted and proper identification carried out. In this case, we used the most generally accepted system of bacterial classification presented in *Bergey's Manual of Determinative Bacteriology*. The preliminary results indicate the occurrence of four species: *Bacillus sp.*, *Pseudomonas sp.*, *Micrococcus sp.*, and *Staphylococcus sp.*

Investigations are underway to determine whether the picture represents a consistent gut flora population or there is a variation with dietary changes brought about by seasonal changes.

The effect of eight phenolic acids previously described from sorghum plants by Conn, Butler (1969) and

Woodhead, Bernays (1978) upon larval survival, growth, pupation and pupal weight of *Ch. partellus* and upon development of bacteria colonies identified in larval intestines is being investigated. The main purpose of this investigation is to estimate the methodology in studies on the indirect effect of any allelochemicals on the nutrition of stem borer larvae.

Because the infestation of sorghum plants by *Ch. partellus* larvae increases with the age of plants, and concentration of phenolic compounds decreases with development of seedlings, the presumption was made that some of the chemicals may affect the development of *Ch. partellus* larvae and/or their intestinal microflora.

The following phenols were included in our experiment p-hydroxy-benzaldehyde (a product of the cyanogenic glycosicle dhuvin, characteristic compound of young sorghum plants), p-hydroxy-benzoic acid, caffeic acid, ferrulic acid, p-coumaric acid, 0-coumaric acid, gentisic acid, vanillic acid, chelidonic acid, geinic acid and chlorogenic acid.

One hundred freshly hatched young larvae are placed into four plastic dishes (or 25 larvae per dish) containing artificial diet used in the ICIPE Insect and Animal Breeding Unit for *Ch. partellus* mass rearing.

Larval survival, growth, pupation and pupal weight of *Ch. partellus* and the development of colonies of identified four species of bacteria occurring in larval intestines are being evaluated in the experiment.

EXPERIMENTAL BASIS OF *MARUCA TESTULALIS* MASS REARING ON NATURAL FOOD

R. S. Ochieng

Studies on biology, ecology and mass rearing of the cowpea pod borer, *Maruca testulalis* have been the centre of our research activities at the Mbita Point Field Station in 1979. Its importance has been underscored by the collaborative work between ICIPE and the IITA on the basis of cowpea resistance to pest attack. To hasten work on this aspect, it has been decided that we do some screening at Mbita using materials available both locally and imported from elsewhere. In this direction the IITA has been requested to cooperate by making available their germplasm material. During the year germplasm material was collected in various areas of Kenya with the intention of screening them for resistance to *M. testulalis*.

Screening of cowpea cultivars and lines to the pod borer attack requires the development of good techniques for mass production of insects at minimum cost and maximum reliability. The cowpea pod borer has not previously been bred in the laboratory in any great numbers. The reason was probably the failure to locate accurately the oviposition site of the pest. Having identified the site of oviposition, it has now become possible to maintain the population of *M. testulalis* throughout the year without break. Previously it had been almost impossible to maintain a population during the dry season. By using flowers and pods as natural diets it has been possible to keep the population through the dry season. No special conditions have been required to do this except a relatively high degree of sanitation. In an attempt to improve on the sanitation the following experiment has been carried out.

Fecunndity of *M. testulalis* females

Investigations on the fecundity of the cowpea pod borer were conducted by using cages 55cm high, 35cm wide and 45cm long covered by mesh screen and containing Petri dish with cotton wool saturated with 10% sucrose water solution, a young cowpea plant growing in the pot and a pair of female and male. Fifteen pairs of *M. testulalis* adults were used in each experiment. Females mostly laid their eggs on cowpea young leaves. The laboratory conditions were not controlled. The temperatures varied between 21°C and 31°C with an average of 28°C. The humidity in the laboratory also varied considerably between 50% RH and 80% RH. There was no control of the photoperiod. The natural variation of day length of 12 hours light and 12 hours darkness was used. Under these conditions at the Mbita Point Laboratory it was found that the ability of individual insects to oviposit

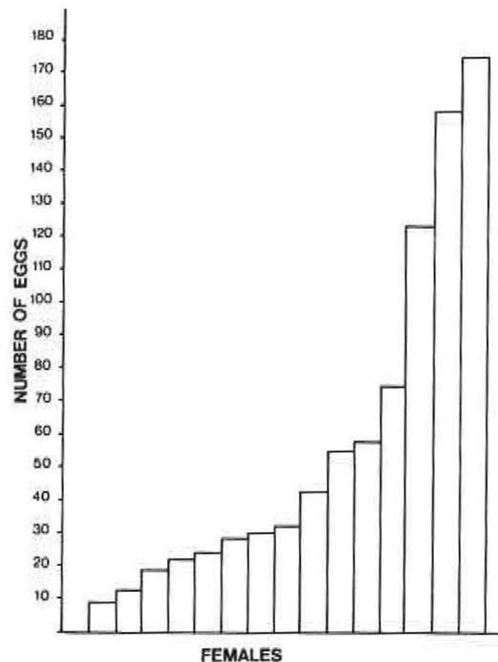


Fig.4 Fecundity of fifteen M. testulalis females reared on natural food under the laboratory conditions of the Mbita Point Field Station.

varied very widely. The range was between 9 and 174 eggs per female with an average of 57 eggs per female. The results are presented on Fig. 4. Half of females tested laid between 30 and 70 eggs. Three females showed high fecundity equal to 120–170 eggs per female. Moths lived for varied periods, ranging from 3 to 18 days, with a mean of 9.5±1.4 for males, and 7.7.±1.0 for females.

Sex ratio of *M. testulalis* population

Studies on the number of adults emerging since the initiation of the laboratory cultures have shown that the sex ratio is 1:1.

Table 4 summarises the results obtained during the past six months.

Table 4. Sex ratio of *M. testulalia* adults from the laboratory colony

Month	Adult emerged		Total	Sex Ratio
	Females	Males		♀ ♂
June	28	31	59	1 :1.1
July	78	59	137	1.3:1
August	53	55	108	1.2:1
September	161	189	350	1.2:1
October	139	165	304	1 :1.2
November	130	131	261	1 :1

Hatchability of eggs

Experiments performed since the establishment of the pod borer colony on the viability or hatchability of the eggs, show that most of the eggs are viable and that no special conditions are required for incubation. The incubation period is between 2–3 days. Results obtained over a period of six months are shown in Table 5.

Table 5. Viability and hatchability of *M. testulalis* eggs from June to November, 1979

Month	No. of eggs collected	No. of larvae collected	Percentage of hatchability
June	1268	807	63.6
July	2307	2305	99.9
August	2584	2512	97.2
September	2299	2005	87.2
October	2429	2382	98
November	3462	2822	81.5

Effect of antimicrobial compounds

The effect of some antimicrobial compounds on the rearing of *M. testulalis* on natural food materials was investigated under laboratory conditions starting in late September. The cowpea flowers were washed in various concentrations of common antimicrobial compounds for 15 minutes. Twenty-five larvae were used in four replicates. The control was washed in distilled water.

Table 6. Effect of some common antimicrobial compounds on survival of *M. testulalis* larvae on natural food materials

% Concentration	Compounds				
	Methyl-p-benzoate	Sorbic Acid	Formalin	Ethyl Alcohol	Control distil. water
0.025	48	20	36	20	40
0.05	64	44	48	12	40
0.10	56	36	8	16	24
0.25	64	36	0	12	20

Table 6 shows that the percentage survival of the larvae during the developmental period up to pupation (duration of 9 days) is increased when the food material is washed in methyl-p-benzoate. With this compound the survival rate was 60% as compared with the control which was between 20 and 40%. Formalin and alcohol solutions showed poor survival percentage at all concentrations with the mortality reaching a peak at the highest concentration of 0.25%. The high mortality of larvae reared on flowers treated with higher concentrations of the last two chemicals in comparison to the mortality of control insects indicates the toxic effect of both compounds.

Microbial organisms are known to be among the major causes of the high larval mortality in *M. testulalis*. The fact that washing the flowers with some antimicrobial compounds can increase the survival by about 20% confirms the observation that microbial organisms are the major causes of mortality in breeding of *M. testulalis*. By using some of the common antimicrobial compounds, this mortality can be significantly reduced. The best of these compounds appears to be methyl-p-benzoate.

Emergence of adults

The larvae obtained during the six months since the establishment of the laboratory colony, have been used in various experiments. During these experiments percentage adult emergence from pupae was determined. This varied from 46% to 95% as shown in Table 7.

Table 7: Emergence of *M. testulalis* adults under laboratory conditions

Month	No. of pupae	No. of adults emerged	Percentage of emergence
June	62	55	95.0
July	262	123	46.0
August	240	110	45.8
September	395	347	87.7
October	388	303	98.0
November	296	280	94.6

Present efforts dealing with improvement of techniques of handling larvae may eliminate the need for applied manual transfer of larvae in two-day intervals from the cages with used food to cages with fresh food materials.

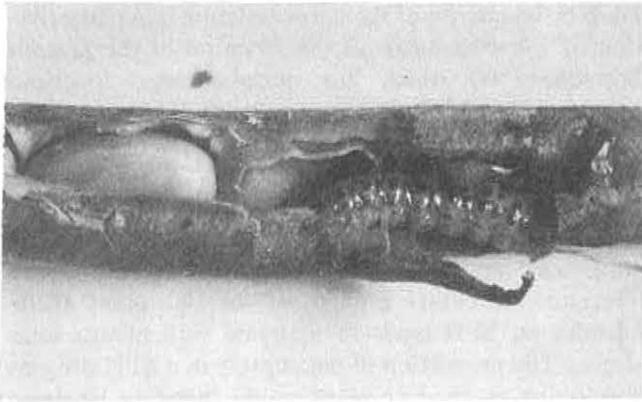


Fig. 5. Larva of *M. testulalis* rearing on cowpea pods under laboratory conditions.

The larval negative phototaxis will be explored in this new method (Dabrowski and Ochieng).

Studies on female oviposition behaviour on different artificial surfaces covered by cowpea leaf fractions are conducted with the aim to simplify the present procedure of egg collection from leaves of potted cowpea plants (Dabrowski, Gebreyesus, Ochieng). The active chemical compounds could be classified later as attractants. The experiments now being conducted on larval feeding behaviour may indicate existence of feeding incitants and stimulants in cowpea flowers, leaves, pods and/or stems. Identification of these chemical compounds allows us to start extensive work on *M. testulalis* mass rearing on artificial diet.

ICIPE—IRRI Staff

Ramesh C. Saxena—Research Scientist
Nicanor J. Liquido—Senior Research Assistant (up to August 1979)
Belen C. Puma—Research Assistant
Hilario D. Justo—Research Aide
Rosalito L. Villanueva—Secretary

Bases of resistance in rice varieties

R. C. Saxena

Relative susceptibility or resistance of rice varieties and the common barnyard grass to the brown planthopper (BPH), *Nilaparvata lugens* (Stål), is determined by an interaction of the insect responses to the plants and effects of plants' physical and chemical stimuli on the insect. Previous studies had indicated that rice varieties and barnyard grass possessed characteristic odours which influenced the hopper's orientation to these plants. Since upon arrival the insect also comes in contact with the plants' volatile components such as essential oils, we tested the effects of the steam distillate extract from leaf sheaths of Biotype 1-resistant Mudgo, ASD7, and IR26 varieties, susceptible IR20, IR8, and TN1 varieties, Ptb33 which is resistant to three BPH biotypes at IRRI, and the nonhost barnyard grass.

Differential mortalities of BPH biotypes were recorded on topical application of the essential oil extracts of test plants. Biotype 1 was highly susceptible to extracts of all resistant varieties and barnyard grass, as 20 to 80% females died at doses of 5 to 20 µg/female and almost all died at the dose of 50 µg/female. IR26 extract caused high mortality only at 50 µg level. Few insects died at even high doses of IR8 and TN1 extracts but extract of IR20 was fairly toxic at 50 µg level. Biotype 2 suffered high mortality with extracts of barnyard grass, Ptb33, and ASD7. Insect mortality was low at lower doses of extracts of Mudgo, which is susceptible to Biotype 2, but increased at higher doses. Biotype 2 mortality with extracts of IR8 and TN1 was much lower than that caused by IR20 and IR26 extracts. Biotype 3 suffered relatively higher mortality with extracts of Mudgo, IR26, and IR20 than that by ASD7, IR8, and TN1 extracts. Toxic effects of Ptb33 and barnyard grass extracts, however, were not as pronounced as those on other biotypes.

The quantity of essential oils among Mudgo, ASD7, IR26, IR20, IR8 and TN1 varieties ranged from 46 to 59 mg/200 g fresh leaf sheath. Yield of essential oils was markedly higher, ca. 82 mg/200 g leaf sheath from the traditional variety Ptb33, which is resistant to all the three BPH biotypes, and ca. 107 mg/200 g leaf sheath from nonhost barnyard grass. Insect's differential survival on exposure to volatile components of the test plants indicated that the rice varieties and barnyard grass not only differ quantitatively but also in the nature of their essential oils.

The role of nutritional factors (particularly amino acids, which are a major source of BPH nutrition) in relative susceptibility or resistance of selected rice varieties to BPH was investigated. A comparison of the profiles of seven common rice amino acids-alanine, asparagine, aspartic acid, glutamic acid, leucine, serine, and valine, showed that their content differed in Mudgo, ASD7, and TN1 varieties. Bioassay of these amino acids showed that for Biotype 1 asparagine and valine were highly phagostimulatory at 4% concentration while alanine and serine were moderately phagostimulant. For Biotype 2, alanine at 4% concentration was most phagostimulatory, while valine, serine and asparagine were moderately phagostimulatory in that order. For Biotype 3, valine and serine were most phagostimulatory followed by asparagine and alanine. Leucine

was more or less inert for the three biotypes while aspartic and glutamic acids were inhibitory at higher concentrations. The content of asparagine and valine, which were most phagostimulatory to Biotype 1, was higher in TN1 than in Mudgo. Alanine, most phagostimulatory to Biotype 2, had a higher content in Mudgo than in ASD7 or TN1. Valine and serine, highly phagostimulatory to Biotype 3, had a higher content in ASD7 than in other varieties. This indicates a chemosensory specificity of the three BPH biotypes for different amino acids in the three rice varieties.

Host plants' internal chemistry is also likely to influence hatching of BPH eggs, which on oviposition incubate inside the ruptured leaf sheath parenchyma. Since previous studies had shown reduced hatching on resistant rice varieties and on nonhost barnyard grass, we considered the effect of *trans*-aconitic acid (t-AA), a major barnyard grass allelochemic, on BPH hatching. Hatching of BPH eggs, irrespective of biotypes, was low when t-AA concentration was high and solution acidic; in a 1% solution at 2.7 pH, only 4–13% eggs hatched. Adverse effects on hatching occurred even when the t-AA solution was neutralized with calcium hydroxide, particularly at 0.1, 0.5 and 1% concentration. In barnyard grass, t-AA concentration is about 0.5% of the fresh plant weight. Immersion of BPH eggs in 0.5% t-AA solution for 3 days or longer reduced their hatchability much more than did immersion for 1 or 2 days, indicating the susceptibility of the developing BPH embryo to the chemical environment at 3 days after oviposition. This developmental stage probably coincides with the disappearance of the vitelline membrane which in insects is generally resistant to chemicals. Studies are in progress on the major organic acids found in susceptible and resistant rice varieties and their effects on BPH hatching.

The above studies tend to show that allelochemic factors in combination with a proper nutritive balance in plants are necessary to obtain optimal insect-plant interactions. Plants are resistant to an insect species if they possess allelochemic factors which do not evoke positive insect response, and exert adverse effects on the behaviour and physiology of the insects if they are confined on resistant plants. An optimum nutritional balance is of course a pre-requisite for normal growth, survival and reproduction.

Wing morphism in brown planthopper

The changes in the physiological status of the host rice plant were found to have a profound effect on BPH wing morphism. Significant increase in macroptery in BPH progenies reared on senescent or "hopper-burned" hosts was because of a general decline in the host plant's nutritional status and a change in the level of allelochemic factors. Continued stress of starvation perceived by BPH nymphs when restricted to feeding on nutritionally-depleted, senescent rice hosts probably inhibits the activity of the corpus allatum (CA). Inactivation of CA then turns off the secretion of the juvenile hormone (JH) which has morphogenetic functions during nymphal life. In the absence of JH, brachyptery is suppressed and macroptery becomes operative. The host plant's allelochemic factors may have a direct bearing on wing morphism. Green tissues of young rice plants are rich in chemicals that mimic JH (JHm), while senescent plants are deficient in these substances. Therefore, juvenilizing effect of the rice plant allelochemics on BPH tends to dissipate with plant's senescence. The proportion of macroptery in a BPH progeny developing on the rice plant would therefore be determined by the extent of nutritional depletion and the decrease in the level of JHm substances in the host plant. Crowding during BPH development affects the host plants which in turn affects BPH wing morphism. BPH wing morphism is thus an expression of plant-insect interaction and represents the insect's adaptation to a changing but nontheless predictable environment.

Long distance BPH migration in Philippines

Migrating insects were caught on two successive inter-island voyages abroad Escaño Lines Cargo-passenger ships MV SURIGAO and MV AGUSTINA II in the territorial water of the Philippine Archipelago during 1979 wet season, which is characterized by the South-west Monsoon from the Indian Ocean. BPH, WBPH, and other rice hoppers were mostly caught on the Manila-Cebu, and Cebu-Manila routes, which lie in the path of the Southwest Monsoon—a warm and very moist air mass that flows constantly towards the Philippine island during the wet season. These air currents are ideal for transporting insects over long distances. Thus migrant hoppers and other small insects may be carried to the Philippine Archipelago by air currents from certain rice-growing areas lying to the southwest of the Philippines. Exploratory voyages for monitoring migrant hoppers during other seasons are being planned.

BPH flight activity—Invasion

We studied the invasion flights of BPH into rice fields. Comparison of cumulative hourly catches of immigrants in yellow pan traps (YPT) and on yellow sticky board traps (YBT) showed that maximum number of BPH macropters was recorded in the early morning catch at 6 a.m., indicating that invasion flight activity peaked around sunrise. Catches of hoppers and their number on 1- or 2-m-high sticky traps were identical, suggesting that height of traps at 1 or 2 m above the ground did not change their trapping efficiency.

Daily monitoring of hoppers through 40 trapping days, based on cumulative number of hoppers in catches in 128 YPTs or on sixteen, 1- or 2-m-high YBTs showed that immigration activity occurred mostly during the vegetative stage of crop growth.

Extract of an indigenous plant for control of brown planthopper and other crop pests

We have started a systematic search of commonly-occurring, pest resistant plant taxa, and extraction, isolation and identification of their chemical principles with a view to utilizing them in integrated pest control programmes. Since these chemicals already occur in nature and are mostly biodegradable, their use is expected to have the least disruptive effect in the environment.

We found that extract of an indigenous plant was highly effective against numerous insect pest. Even extremely small quantities of the extract repelled insect pests, reduced their feeding, disrupted embryonic and larval growth, and decreased survival and oviposition.

We also tested the extract against common pathogens of rice and found that it inhibited the growth of causal organisms of the bacterial leaf blight, leaf streak, leaf rot, and wilt, and the fungal leaf scald. leaf spot, blast and sheath rot. Further tests against the rice blast fungus showed that the extract inhibited the mycelial growth and inhibited spore germinations. The extract did not affect the causal organisms of Bakanae and leaf spot.

The plant extract had negligible or no toxicity on the predators such as spiders, mirid bugs, and *Microvelia*, commonly occurring in rice fields.

Field sprays of the plant extract reduced BPH nymph and adult population, as compared to that in the control plots.

Effect of neem seed oil on leaf folder

Seed oil of the widely-distributed neem tree was found to be highly effective against the rice leaf folder from egg to adult stages. In leaf studies larvae were repelled and their feeding significantly reduced when offered leaves were sprayed with 12% or higher concentrations of emulsified, crude neem oil. Oviposition was also deterred as the leaf folder moths laid only about one-third the number of eggs on plants treated with neem oil than on plants sprayed with water. Hatchability of eggs on treated plants was only 25 to 50%, compared with about 80% eggs hatched on control plants.

Neem oil had a synergistic effect on parasitization of leaf folder larvae. Rice fields sprayed with 50% neem oil had about 35% larval parasitization, nearly twice as high as that in control plots.

Neem oil's antifeedant property, however, is rapidly degraded by sunlight if relatively weak and unstable formulations are used.

CROP BORERS RESEARCH PROGRAMME

Sorghum Shootfly Project
Visiting Director of Research
Professor K. N. Saxena (1978)
Programme Leader
Professor E. H. Smith (1979)

Research Staff
Mr. G. O. Amala (1976) Subordinate Assistant
Mr. G. M. Bizoza (1977–79) Principal Technician
Mr. A. G. L. Delobel (1978) Research Scientist
Mr. K. E. Kidega (1977) Senior Technician
Mr. J. G. Kibuka (1978) Junior Technician
Mr. J. Lumuli (Jan—Nov. 1979) Technical Assistant/ Driver
Mr. J. Marasa (1979) Subordinate Assistant
Mr. K. Ogwaro (1973) Scientific Officer
Mr. J. C. Olela (1977) Senior Technician
Mr. G. E. Oloo (1979) Subordinate Assistant
Mr. S. M. Othieno (1973) Technician
Dr. A. K. Raina (1977) Research Scientist
Dr. G. C. Unnithan (1978) Research Scientist
Dr. S. Yagi, Visiting Scientist

Collaborators
Mr. J. Owor, Histology and Fine Structure
Mr. Isaac Jondiko, Chemistry/Biochemistry
Dr. D. Whitehead, Chemistry/Biochemisty
Dr. Z. T. Dabrowski, Bases of Plant Resistance

Introduction

Crop Borers Research

E. H. Smith

The term Crop Borers as used herein refers to insect pests of graminaceous and legume food crops of Africa. The group includes diverse species, a Dipteran, the sorghum shootfly *Atherigona soccata* which is restricted to sorghum, millet and several wild host plants. Several lepidoptera, the pod borers *Maruca testulalis* and *Heliothis armigera*, major pests of grain legumes. Stem borers, the spotted stem borer, *Chilo partellus* which has a wide range of host plants including maize, sorghum, millet, rice and sugar cane; the maize stalk borer *Busseola fusca* on maize and sorghum, the pink stalk borer *Sesamia calamist* on maize, sorghum, millet, rice and sugarcane, and the rice stem borer *Maliarpha separetella*, a major pest of rice. The crop borers often occur as a complex of pests and their identity and ecology has been a matter of much confusion.

This group of pests is of special significance to tropical Africa for several reasons. First, they attack the major food staples of Africa. While in many parts of the world, wheat and rice are the major staples, this is not the case in Africa; maize, millet, sorghum and cowpeas being the staples. Indeed, over 60% of the cultivated land devoted to food production in Africa is planted to these crops. In several countries, including Kenya, a single crop, maize, accounts for over 50% of the food energy. This brief overview highlights the critical importance of a pest such as the spotted stem borer (*Chilo*) which attacks most of the major food crops of Africa.

Secondly, these staples are the major food crops of subsistence farmers whose operations are characterized by small size and intercropping. Both the level of technical proficiency of the farmers and their traditional practices of intercropping pose special problems. Little research and educational effort has been addressed to this system of agriculture. The thrust of the Green Revolution was under conditions of monoculture and large plantings. The limits of the technology of the Green Revolution have become increasingly apparent in recent years and subsistence farming has loomed as the great challenge in improving the food supply in developing countries. While the complexities posed by subsistence farming are staggering, there is cause for optimism. The subsistence farmer has been found to be far more receptive to improved methods than had been assumed. The striking gains with hybrid maize in Kenya led to the conclusion by Wortman and Cummings that '. . . . Kenya has shown that small subsistence farmers, given the opportunity, will adopt appropriate technology almost as rapidly as large commercial farmers". Surely insect ecologists will find exciting challenge in unravelling the ecological relationships underlying the conventional wisdom of subsistance farmers in their inter-cropping system and making further advances in the context of modern pest management. Indeed, this challenge represents the new frontier of insect pest control in tropical regions of the world

The strategy of the Crop Borers research programme reflects the influence of the factor cited above. Simply stated the strategy is to (a) give highest priority to major food crops and their pests (b) determine the ecological relationships involved in traditional subsistence intercropping (c) obtain specific information on ecology

of pest species as the long range foundation for effective pest management (d) establish relationships with subsistence farmers to serve as a two way bridge for the flow of information and testing of research findings. The reports which follow are best viewed as sub-units of the larger research design.

Sorghum Resistance to Shootfly and Shootfly Behaviour

A. K. Raina

Sorghum Resistance to Shootfly

Two international shootfly nurseries have been received from ICRISAT, and planted at Mbita Point field station. The results from the 1978 nursery were compiled and sent to ICRISAT. The 1979 data are being compiled at the time of writing. Both nurseries did very well and yielded excellent data. In addition to shootfly the entries were also evaluated for stem borer resistance.

Several promising lines were selected each year and replanted in replicated trials. Based on oviposition and dead heart counts for shootfly taken on the 14th and 28th day after germination, 5 lines (IS 1054, IS 1082, IS 2195, IS 2312 and IS 3962) were selected in 1978 and 2 lines (IS 2146, and IS 5613) in 1979. Percent dead hearts in the 1979 trial ranged from a low of 7 in IS 2146 to 77 in CSH-1. An interesting feature about some of these lines was a high ratio (3:1) between the plants with eggs to plants with dead hearts. All these 7 lines are now being used in several experiments to determine the mechanisms of resistance. On the basis of data from field and laboratory observations 4 lines (IS 1082, IS 2146, IS 3962 and IS 5613) were selected and planted along with "Serena" the recomended East African variety, in a field trial to make a final evaluation for resistance and agronomic qualities. Data on shootfly infestation; are presented in Table 1. All the 4 lines proved very good for shootfly resistance, particularly IS 3962.

Table 1. Shootfly data in the entomology/agronomy trial with selected cultivars of sorghum—1979*

	14 days			28 days		
Cultivars	Total No. of plants	% plants with eggs	% plants with dead hearts	Total no. of plants+ tillers	% plants+ tillers with eggs**	% plants+ tillers with hearts
IS 3962	155	3.87	0.00	303	2.31	5.28
IS 5613	172	8.93	1.20	222	3.15	3.15
IS 2146	167	9.13	1.23	283	3.18	4.24
IS 1082	173	11.78	6.20	253	3.16	9.88
Serena	172	33.35	17.23	514	17.90	29.57

*Four replicates, each plot made up of 5, 2 metres rows. Data taken from middle 3 rows.

**Some of the eggs could have been washed or covered with mud in which case the dead heart counts are more valid.

In single choice cage experiments conducted with 6 selected cultivars, a local variety "othuwa", and CSH-1 as control, oviposition was significantly less on IS 1082, IS 2146, IS 2195 and IS 3962. IS 1082, IS 2146 and IS 3962 also received significantly fewer eggs per plant.

Growth of the larvae on various cultivars was determined by making daily measurements of size (length and width) and weight from hatching to pupation. Larvae reared on IS 2312 and IS 3962 were significantly smaller and weighed less than those reared on control variety.

When fecundity of the flies reared on various cultivars was tested on CHS-1; flies from IS 1082 and IS 3962 had a longer preoviposition period (3.8 and 3.5 days respectively compared to 3 days for control). Fecundity was significantly reduced in case of flies from IS 3962 and IS 2195. There was no significant difference in the egg hatch. Female longevity was lowest when reared on IS 2195. These studies are being continued by Miss H. Thindwa a graduate student.

Shootfly Behaviour

Larval movement, growth and feeding behaviour

The study was done at 30°C on CHS-1; a susceptible variety of sorghum. Shootfly eggs are laid on the underside of the leaves and hatch early in the morning. The young larva moves through the water film from the morning dew to reach the base of the leaf sheaths (Fig. 1). It then penetrates the central shoot and girdles it, causing the typical dead heart symptom. The first instar which lasts 1–3 days seldom damages the growing point. The second instar feeds extensively on the central shoot and lasts 1–3 days. Most of the growth takes place during the third instar, which feeds mostly on dead and decaying tissue. The mouth hooks change from 2 pairs in the first and second instar to a single pair in the third instar. The mature larva is about

6–7 mm long and contains a large quantity of deep yellow fat. Under normal conditions of moisture the larva pupates in the soil. However, if the plant and soil are dry, it descends down to the base of the plant and pupates in a cell with its anterior end up.

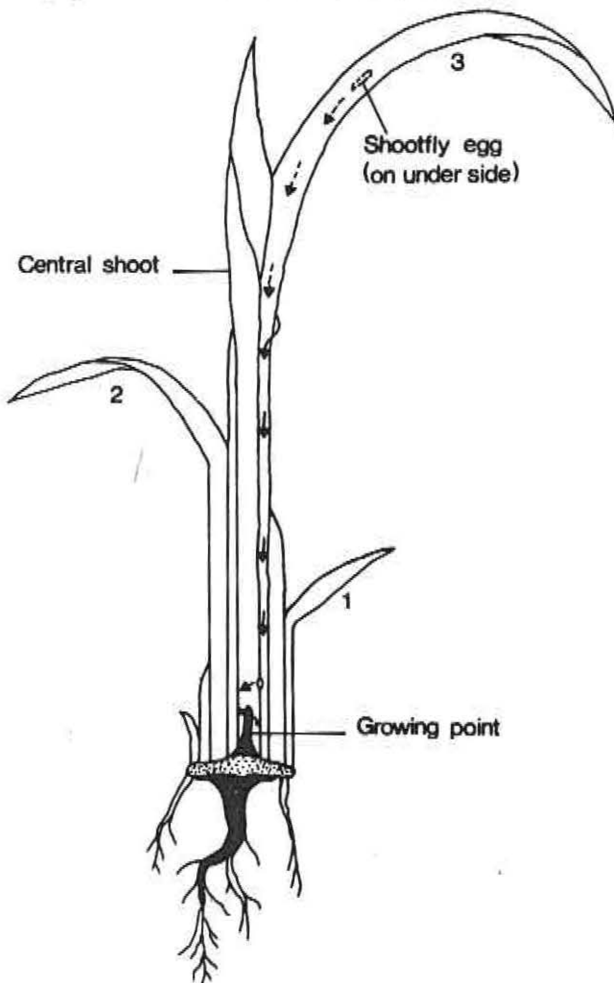


Figure 1. Path followed by a 1st instar shootfly larva from the site of its hatch to the base of the sorghum plant, where it penetrates in and commences feeding.

Even though several eggs may be laid on a single plant, only one larva survives in it, the rest being killed by the most vigorous one. This cannibalistic behaviour does not occur outside the plant host (when placed on artificial diet), no matter how crowded the larvae are. The first instar larvae are not capable of migrating from one plant to another, but the third instars may do so if the plants are not big enough to sustain these to maturity. Movements of the freshly hatched larvae to the base of the plant is not due to positive geotaxis, as plants kept upside down were also successfully infested. However, when the plants (in pots) were kept horizontally, the larvae confined to the upper region and their growth was much slower.

Fish-meal as shootfly attractant

Fish-meal has often been used in shootfly traps. Two disadvantages with the fish-meal are that it is not specific for sorghum shootfly and that predominantly more females are caught in such traps. When 2 groups of 0 to 6 day old females (one provided with brewers yeast and glucose and the second with glucose alone) were tested in a behaviour chamber for attractancy to fish-meal, it was noticed that attraction to fish-meal was greatest immediately after emergence and after laying a batch of eggs. Gravid females showed very little attraction to the fish-meal (Table 2). This was particularly true of flies fed only glucose. This experiment suggests that the females are attracted to the fish-meal because of their need for protein.

Table 2. Percent female shootflies visting the fish-meal and control sectors of the test chamber during a 30 minute period*

Age of test adults in days	Females prior to the test provided with brewers yiest, glucose, water plants and males		Females prior to the test provided with glucose, water, plants and males	
	Fish-meal	Control	Fish-meal	Control
0	19.9±5.8	16.7±3.3	21.7±2.9	7.2±2.5
1	17.2±3.5	19.4±1.9	14.9±1.8	22.8±4.2
2	17.8±3.5	13.9±3.5	17.8±6.3	13.7±4.8
3	13.9±4.2	25.0±6.0	11.1±3.5	16.7±4.4
4	27.8±3.9	18.9±5.9	16.1±4.8	12.2±2.5
5	14.4±4.2	15.0±2.9	18.3±2.9	16.6±2.9
6	25.0±8.3	7.8±4.2	11.1±2.5	10.5±2.5

*Based on an average of 3 replicates, with 4 females in each replicate; ± standard deviations.

Oviposition marking and deterring pheromone

It was observed and has also been reported by earlier workers that under low infestations, the shootflies lay only one egg per plant. There are two possible mechanisms by which this is regulated; by visual recognition of the existing egg or by means of a chemical marker. Three experiments were set up to investigate this phenomenon.

The shootfly attaches its eggs to the underside of the leaves by a water soluble glue. In the first experiment, eggs washed thoroughly in distilled water were attached by paper glue, one per leaf, to all the leaves of alternate plants in a tray. A gravid female was caged over each tray and the eggs on all plants counted after 24 hours. In the second experiment, flies were allowed to lay a large number of eggs on few plants. The leaves with eggs were then removed and shaken in distilled water until the eggs were detached, and then filtered. The filterate was sprayed on alternate rows of plants growing in trays. The control rows were sprayed with water in which leaves without eggs had been washed. A gravid female was then caged over these plants and egg distribution recorded. In the third experiment flies were allowed to lay eggs on test plants, which were then removed by a moist camel-hair brush. These plants were then presented to gravid females along with plants on which no eggs had been previously laid. The results are presented in Table 3. The oviposition response did not differ significantly in the first experiment where washed eggs were placed on leaves of test plants. This indicates that the females are not deterred by the sight of an egg. In the other two experiments the differences were highly significant. This strongly suggests that some sort of a chemical deterrent is present, perhaps in the adhesive substance of the eggs.

Further tests with this chemical are in progress in cooperation with the Chemistry/Biochemistry research unit.

Table 3. Results of experiments to check the possibility of an oviposition marking pheromone in the shootfly

	Visual Recognition		Chemical marking		
			Egg-wash spray		Eggs removed
	% plants with eggs	No. of eggs/plant	% plant with eggs	No. of eggs/plant	Average no. of eggs/plant
Treatment	70.0	1.76	54.4*	0.91**	3.1**
Control	77.5	1.33	72.2	1.60	13.0

*significantly different at P=0.05, and ** at P=0.01.

Sorghum Shooftly: Reproducttive Biology; off-season Survival; and effect of precocene

G. C. Unnithan

Reproductive Biology

Frequency distribution of ovarioles:

Laboratory studies have revealed a wide variation in fecundity of shootflies. In order to ascertain if there is any anatomical basis for this variation, frequency distributions of ovarioles in 200 nulliparous females were determined (Fig. 2). The number of ovarioles ranged from 20-43 with a mean of 32.5±4.3 ovarioles per female. 23.4% of the total ovarioles from 176 females (mean 7.6±5.4) appeared to be "non-functional" or at least they did not mature any oocytes during the first few days. Apparently the variation in fecundity could be partly due to the variation in the number of ovarioles and the presence of non-functional ovarioles. Preliminary studies indicate that adult nutrition influences the number of ovarioles with oocytes undergoing vitellogenesis (functional ovarioles). Females fed on honey dew, from aphid-infested sorghum seedlings, instead of feeding on the laboratory diet of 1:1 glucose and yeast, showed an acceleration of egg maturation as well as an increase in the number of functional ovarioles. Out of 16 flies fed on honey dew 15 had gravid oocytes in the ovary when they were two-day old; whereas only 5 out of 15 flies fed on glucose and yeast had, gravid oocytes. 5.4% and 13.7% of ovarioles were nonfunctional in the honey dew fed and glucose-yeast fed flies, respectively. Further studies on the role of nutrition on egg maturation are in progress.

Effect of mating and presence of males on fecundity of shootflies

Fecundity, egg hatch, preoviposition period and longevity of three groups of insects were determined. These are: (i) virgin females; (ii) males and females kept together; and (iii) females allowed to mate only once. The flies were maintained at 30±1°C, 60±10% RH, and 12:12 LD regimen and fed on 1:1 glucose and yeast. The results are shown in Table 4 (1-3). Lack of mating delayed and/or inhibited egg-laying. None of the eggs laid by the virgin females were fertile. There was no significant difference in longevity among the 3 groups. Fecundity and egg hatch of the females kept with males, and of females allowed to mate only once, also did not differ significantly. It is evident that a single mating insures fertile eggs by the female, indicating that sperms can be stored for prolonged periods of time.

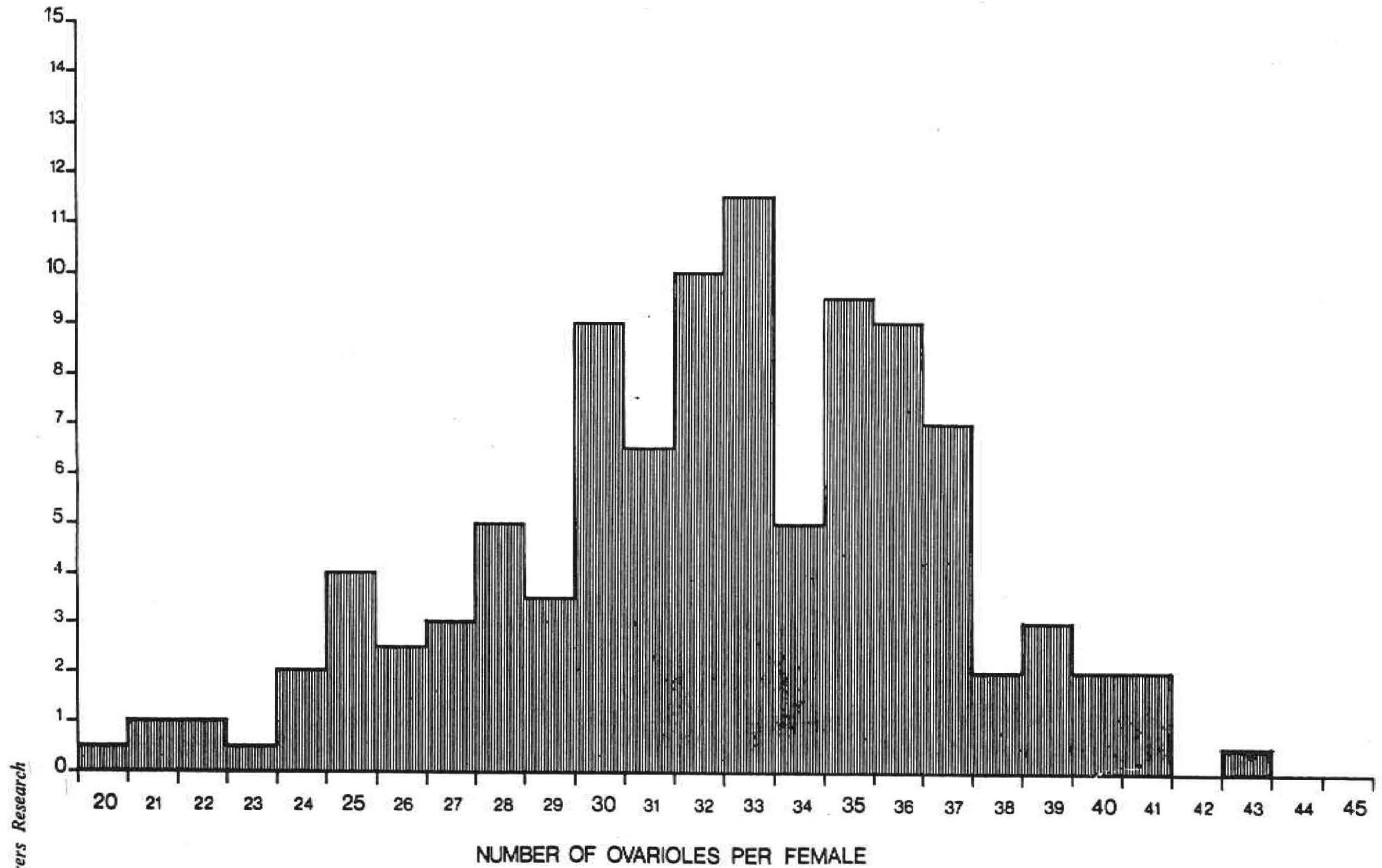


Figure 2. Frequency distribution of ovarioles; based on counts from 200 nulliparous females.

Fecundity of off-season (dry season) shootflies

Reproductive potential of off-season (dry season) shootflies is an important factor in the initial infestation of sorghum at the beginning of the growing season. The fecundity of off-season females was determined from data on fecundity, egg-hatch, preoviposition period and longevity of shootflies from larvae and pupae obtained from farmers' fields at Mbita and Emali and from wild sorghum, during the dry season (March, 1979 and July-September, 1979), under laboratory conditions. The data are given in Table 4. There was no significant difference in the fecundity of these flies ($P > 0.30$) when compared to that of flies which were not subjected to stress of dry weather (regular colony). However, the preoviposition period and longevity of the off-season flies were significantly higher ($P < 0.02$) when compared to those of flies from regular colony.

Fecundity of flies subjected to dry and wet conditions during larval and pupal period

Fecundity of insects whose larval and pupal development were completed in seedlings maintained under dry and wet conditions in the green house were studied. In one case the infested seedlings were watered daily and in the other they were not watered and began to wilt after infestation. No significant difference was observed in fecundity and longevity of flies subjected to wet conditions when compared to those of flies subjected to dry conditions during third larval and pupal period (Table 4, 4–6). However, the preoviposition period in flies subjected to wet conditions was considerably higher ($P < 0.001$).

Mating behaviour: influence of adult age, and removal of trifoliate organ on mating

Shootflies normally start mating 1 day after emergence. However, mating rarely occurs on the day of emergence, if the flies are maintained at high temperature. The number of flies mating increases with increased age, perhaps reaching a peak in three-day old insects (Table 5). Females normally mated only once. Males mated several times during their lifetime with virgin females.

The trifoliate organ (trifoliate process (TF) on the 8th segment of the male) is employed in courtship behaviour. Grasping the females during copulation does not seem to be the function of the TF. Males whose TF were removed also mated successfully (Table 5) although the courtship period was prolonged considerably. There was no difference in the time the pair spent *in copula*, between normal pairs and those where the males were without TF; mating lasted for 26.5 ± 5.9 min. and 25.9 ± 6.2 min. respectively, in these two groups (number of observation: 100 for normal; and 22 for males without TF). Further studies are in progress to determine the function of TF and the presence and role of any sex attractants, and the influence of age and oocyte maturation on the receptivity of males and females.

Off-season survival/aestivation-diapause

Preliminary laboratory and field observations showed no evidence for the suggested occurrence of aestivation-diapause as a mechanism of off-season survival. Larvae and pupae collected during the dry season showed no sign of diapause. These larvae and pupae either died or completed the development as usual, when maintained at dry conditions. In green-house experiments where the larval and pupal development was completed in seedlings subjected to either dry or wet conditions, developmental time of the insect from egg to adult was 23.7 ± 3.5 in seedlings subjected to dry conditions and 29.8 ± 7.8 in seedlings subjected to wet conditions (number of observations 44 and 62, respectively). This difference in developmental period is significantly different ($P < 0.001$). However, when a somewhat similar experiment was done in the field at Mbita Point there was no significant difference in developmental time from egg-adult, within seedlings subjected to wet conditions when compared to that within seedlings subjected to dry conditions.

Fortnightly surveys (in collaborations with A.G.L. Delobel) of shootfly population on cultivated sorghum and on wild sorghum, *Sorghum arundinaceum* are continuing at four locations (Emali, Embakasi, Kenyenya and Kisui) and at two locations (Emali and Embakasi), respectively. These surveys indicate that shootfly population survives at very low level during the dry period (sorghum off-season). Further studies are necessary to confirm the presence of diapause, if any, in the shootflies.

Effect of precocene 1 and 2 on female Sorghum Shootflies

Precocene 1 and 2 isolated from the plant *Ageratum* are shown to be highly potent antiallatotropic substances which induce precocious metamorphosis and sterility in several species of insects. In order to determine the effect of precocene on shootflies, 0–1 day old female shootflies were treated with precocene 1 and 2 either topically or by contact method. For controls, flies were treated with acetone which was also used as solvent for precocene. Both precocene 1 and 2 induced very high mortality (Table 6). There was no inhibition of egg maturation in the surviving flies. No attempts were made to see whether precocene affected the corpus allatum of the flies. It appears that precocene does not have any antigonadotropic activity in shootflies

Table 4. Fecundity and Longevity of *Atherigona soccata* under different experimental conditions

	Treatment	No. of insects used	Longevity in days Mean±S.D	Age in days at the time of first oviposition Mean±S.D	Eggs/female Mean±S.D.	Percent eggs hatched
1.	Virgin females (Regular colony)	22	14.9±6.9	9.8±4.7	7.1±12.4	0.0
2.	Males and females kept together (Regular colony)	19	16.1±7.4	3.1±1.0	57.6±28.8	76.3
3.	Females allowed to mate only once (Regular colony)	20	17.6±10.8	3.6±0.7	74.0±34.5	79.5
4.	Off-season (dry season) shootflies	42	22.9±15.1	4.2±1.9	49.9±29.9	81.1
5.	Subjected to wet conditions during larval and pupal period	10	42.3±29.6	7.0±6.1	73.3±27.7	74.6
6.	Subjected to dry conditions during larval and pupal period	13	36.8±13.5	3.6±1.8	70.5±35.1	76.4

Table 5. Effect of age, and removal of trifoliate organ (TF) on mating in the sorghum shootfly

Treatment	No. of pairs of insects used	Male age in in days	Female age in days	Number of pairs mated (Percent)
Normal	39	1	1	15.38
Normal	28	2	2	71.43
Normal	19	3	3	84.21
Normal female and TF removed male	22	3	3	18.18
Normal female and TF removed male	17	6–8	3	76.47

Table 6 Precocene-induced mortality in females of *Atherigona soccata*

Compound	Method of application	Dose	Number of insects used	Percent mortality (cumulative) after: 1 day	2 days	3 days
Precocene 1	Topical	10μg	38	68.42	89.47	—
		5μg	34	41.18	67.64	70.58
Precocene 1	Contact	5μ5/cm²	11	100	—	—
		2μg/cm²	10	50	100	—
		1μg/cm²	25	4	28	56
Precocene 2	Topical	10μg	13	46.15	84.61	100
		5μg	89	55.06	65.17	78.65
		2μg	15	6.67	6.67	13.67
Acetone	Topical	1μl	45	13.33	22.22	28.88
(Control)	Contact	—	10	0 0	0.0	0.0

SHOOTFLY ECOLOGY

A. G. L. Delobel

Population Fluctuations

Atherigona soccata population fluctuations depend upon a number of factors, among which availability of suitable hosts and soil and air humidity are the most important. Temperature is a very minor factor in Kenya, but certainly affects populations which are established in the Kenyan Highlands on *Sorghum verticilliflorum*. Rainfall acts indirectly through the growth of the plant and the development of tillers and possibly also directly as a mortality factor of eggs, pupae and adults. Entomophagous insects may also, under certain circumstances and in given environments play an important part in the biocoenosis.

Influence of plant age on infestation

Several experiments were conducted under cages at Mbita Point Field Station and provided data on egg-laying and survival of first instar larvae between hatching and initial feeding as related to plant size and age. Oviposition is low on very young plants, increases and reaches a maximum on 15 to 20 days old plants and subsequently decreases rapidly and is practically non existent on plants which have started initiating their panicle. Survival of first instar larvae is 100% on young plants and slowly decreases on plants older than 20 days. The result is that during the first days after germination, the number of dead hearts increases with attractiveness of the plants: it reaches a peak around the twentieth day and then decreases rapidly because both attractiveness of the plants and survival of the first instar larvae decrease. After 30 days, very few dead hearts are formed. In the case of *S. verticilliflorum*, the wild host of *A. soccata*, the situation is quite different, apparently because the young larvae can establish themselves in old stems, even when panicle initiation has already occurred.

Availability of suitable hosts

During the sorghum off-seasons (August to October and December to April), shootfly populations remain active at a very low level, either on tillers developing from

stubble left after harvest or on *S. verticilliflorum* in areas where it is present.

With the onset of rains, the number of susceptible stems rapidly increases and *A. soccata* populations follow with a slight delay; their increase is relatively slow in early planted crops, because the number of ovipositing females is at that time still very low. Main stems are first attacked but at a low rate (10% on variety CSH-1); as they become progressively unattractive to gravid shootflies, tillers are in turn attacked (the rate of infestation may then reach 20%). As the number of tillers increases, they also become infested (Fig. 3). On crops planted towards the end of the rainy season, results are quite different: both main stems and tillers are subject to a very high infestation (more than 50%). Afterwards, infestation decreases rapidly, as less and less tillers are produced by plants which are weakened by drought.

Influence of air humidity

Adult and egg stages are very sensitive to relative humidity; it is known that adults kept in a dry environment and deprived of water cannot survive more than a few hours; as for the egg stage, the percentage of hatching has been studied under humidity-controlled conditions; 100% hatching has been recorded for relative humidities higher than 87%, while humidities lower than 60% prevent hatching (Fig. 4). Even under very dry conditions (less than 60% RH), embryonic development starts and eggs reach a stage where the sclerotized mandibles are visible through the chorion; death of the embryo occurs at a very late stage, sometimes even after the larva has started moving out of its chorion. The fact that larval emergence naturally occurs early in the morning, when dew is present on the leaves, contributes to survival under dry conditions.

Soil humidity

The location of the pupa (either at the base of the stem or in the soil) depends on plant age at the time of infestation, on the ability of the plant to remain in good condition in spite of larval feeding and on the diameter of the stem at the time of pupation. Depth of pupation does not depend on soil humidity and remains fairly constant (from 0 to 13cm, with a mean of 4 cm), except in very dry or very wet conditions (Fig. 5). The percentage of larvae pupating in the stems has been found to be quite low (between 39% at lower humidities and 0% at higher humidities).

Parasitoids and predators

Several parasitoids attack the different stages of *A. soccata Trichogramma kalkae*, which is an important egg-parasite of the rice stem borer *Diopsis macrophthalma* in Malawi, heavily attacks the eggs of *A. soccata*, specially under fairly cool conditions; two parasites attack the larvae: *Tetrastichus* sp. and an undetermined Chalcid. The pupa is parasitized by several Hymenoptera, among which are two Braconids (*Alysia* sp. and *Opius* sp.) and several other Chalcids. Only the first two are of significant importance: at certain periods of the year, up to 55 to 60% of the Sorghum Shootfly population is affected by them. *T. kalkae* is apparently absent from Lake Victoria basin, a situation which would make its establishment there desirable. Only egg predators have so far been recorded, although there is some evidence that pupae (specially those which are formed in the soil) are also attacked, probably by non-specific predators (carabids, cockroaches, etc.). The commonest egg predator in Kenya is a coccinellid belonging to the genus *Scymnus*.

Spatial distribution of eggs

The distribution of eggs in a recently sown plot where no dead heart has yet formed and very few eggs have hatched follows a Poisson distribution (i.e. a distribution such that the presence or absence of an egg on a plant does not affect the laying of another egg). With increasing egg density (artificially obtained in the insectary), a slight aggregation occurs, leading to a Negative Binomial distribution. From field and insectary combined data, Taylor's power law gives a "b" of 1,14 (Fig. 6); Morosita's dispersion index ranges from 0.8 to 2.4 in the field and from 0.9 to 2.6 in the insectary, also indicating a slight departure from random distribution, which is due to a higher number of plants without any egg and of plants with 2.3 or more eggs than would be expected if the distribution was completely random. This situation may arise because females lay their eggs at random, irrespective of whether or not a plant already bears an egg; it may also be that, if an antioviposition pheromone exists (as reported by Raina, this report) its action is masked by various other factors like heterogeneity among individual stems or among hills.

Trapping adults

The attractiveness of fishmeal (FM) to adults of the sorghum shootfly has been demonstrated by Starks in 1970. Since then, only white-coloured water traps baited with fishmeal have been used; it appears that the yellow (Y) colour attracted more adults than white; followed by green, blue and red. Several designs of yellow and green stripes were less attractive than yellow alone. Y alone caught less females and more males than the usual white FM trap: the sex-ratio ((♂/♀) in Y trap was 0.73 as compared to only 0.08 in FM traps. Another feature of the Y water trap is that it catches much less flies belonging to other species of the genus *Atherigona* than does the FM trap: in an experiment set to study the comparison of FM and Y traps, 80% of the total number of *Atherigona* adults caught in the Y traps were *A. soccata*, while only 23% of the *Atherigona* flies

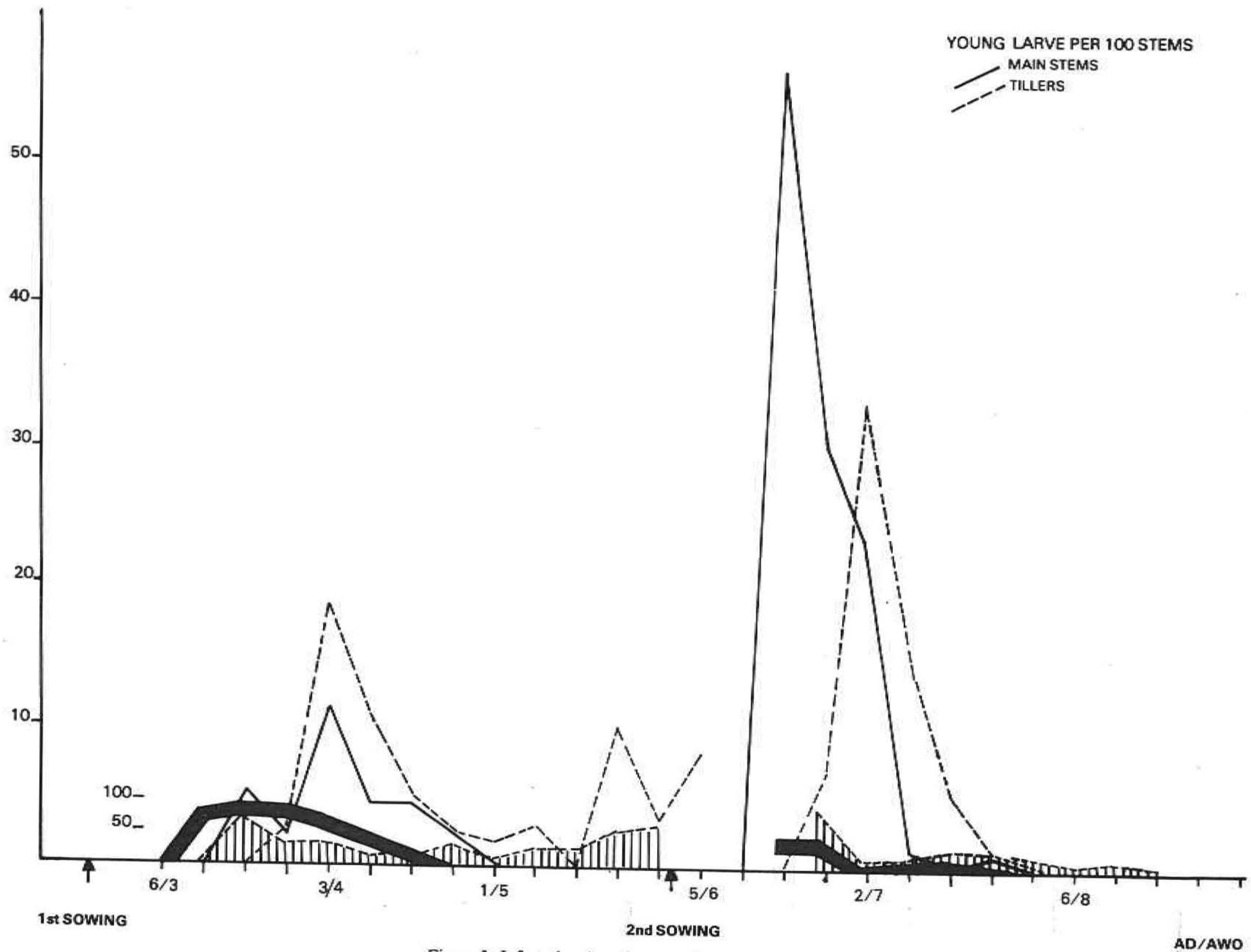


Figure 3. Infestation by *A. soccata* in long rain season sorghum crops (Mbita point). The first plot was sown before the onset of rains, the second after the rains.

The thick line indicates the number of available main stems, the grey area the number of available tillers.

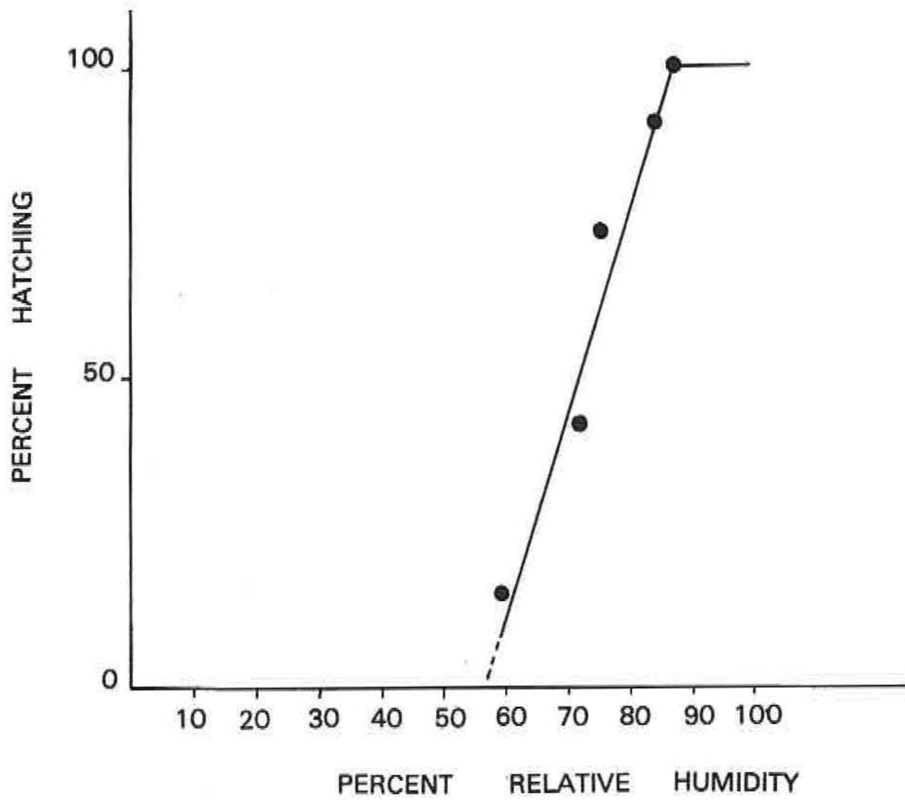


Figure 4. Influence of air humidity on egg hatch in *A. socatta*

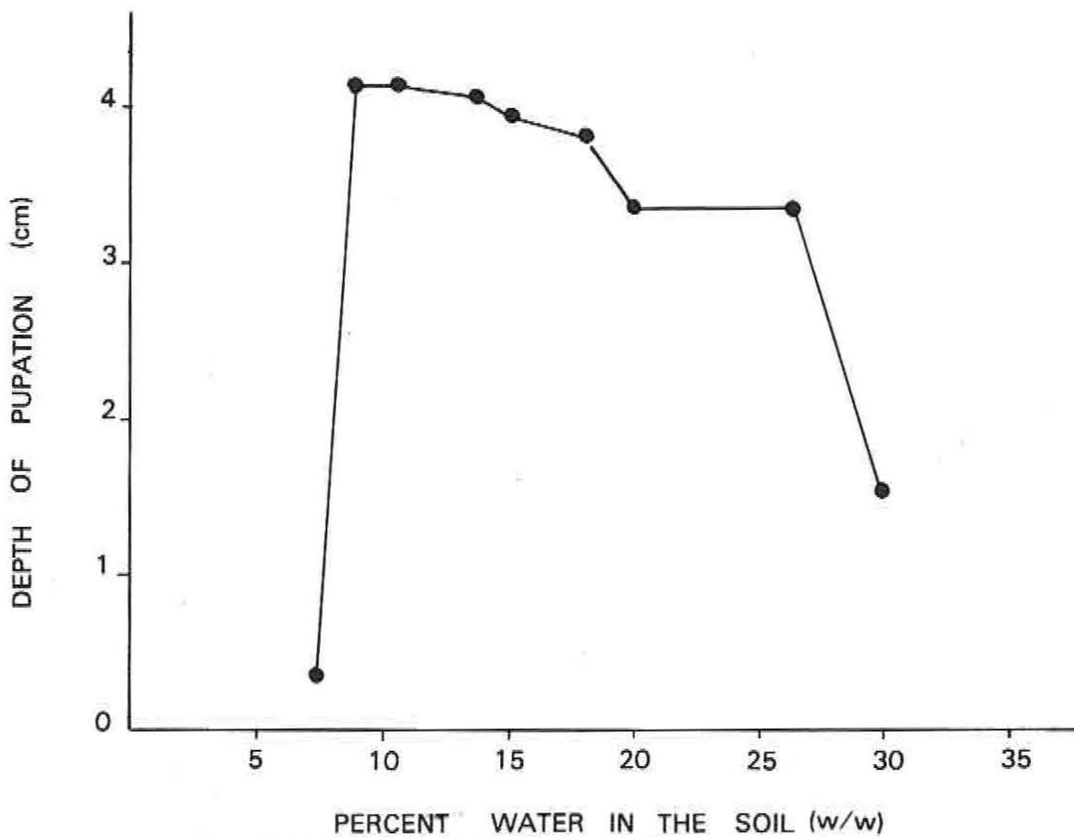


Figure 5. Influence of soil humidity on depth of pupation in *A. soccata*

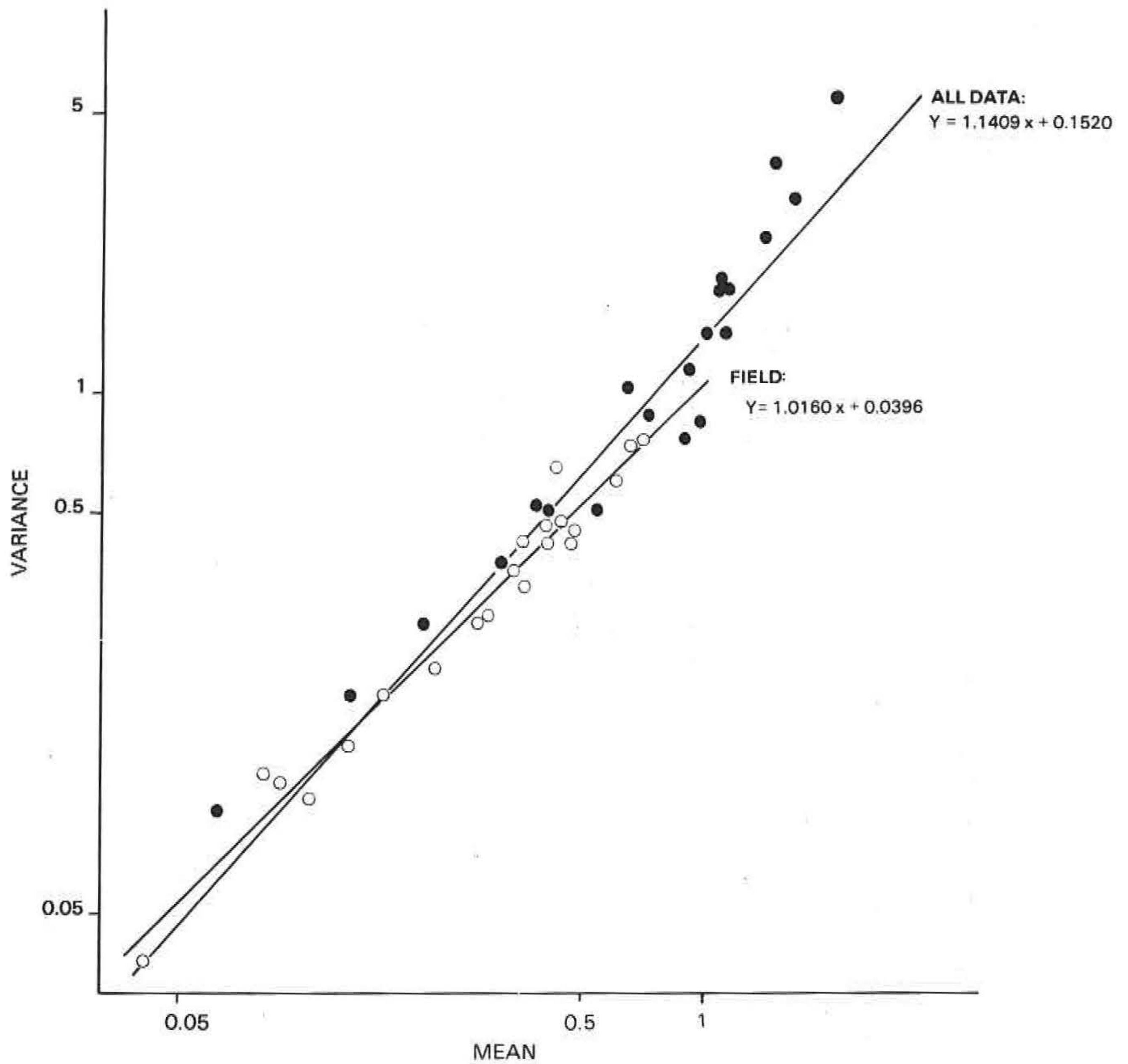


Figure 6. Relationship between mean and variance of *A. soccata* egg numbers in 42 samples of 60 to 100 plants (Field and Insectary data).

caught in the FM traps were *soccata*. This is probably due to a different type of attraction of the two traps: when a trap is set in a sorghum plot, Y catches flies which happen to pass within a very short distance of the water surface whereas FM traps attract by their odour flies which are farther from the trap, including many flies breeding on wild grasses outside the plot.

Addition of yeast extract to the yellow trap improves its effectiveness by two fold. The most efficient trap was a yellow trap (Y) to which fishmeal was added: it caught 3 times more adults of *A. soccata* than the ordinary white fishmeal trap.

As indicated earlier, the position of the trap with regard to the sorghum plot is of great importance: during several experiments in Nairobi, traps set on the margin of or a few metres from a sorghum plot always caught significantly less flies than traps situated inside the plot, indicating that flies probably do not move far when suitable host plants and food are nearby.

Marking adults

Several methods of marking adults of the sorghum shootfly for dispersion studies have been tested, including feeding the larvae with a coloured diet, spraying adults with fluorescent dyes diluted in propionic alcohol and powdering the flies in a screen cage. None of these methods gave satisfactory results, either because excessive handling resulted in high mortality or because marking did not last long enough. A very satisfactory method consists of mixing a water insoluble dye (Methasol and Waxoline dyes by ICI) to the sand in which pupae are placed prior to emergence; very small amounts of the dye remain trapped in the ptilinium and may be revealed after the death of the fly by spraying the appropriate solvent (benzene + acetone + absolute alcohol) on the head. Results indicate that Waxoline green, methasol blue and Waxoline red remain present in the ptilinium during the whole adult life. Waxoline blue and Waxoline orange tend to be absorbed through the cuticule and finally excreted. None of these dyes affected adult longevity.

The pod border *Maruca testulalis* as a pest of cowpeas and studies on its biology

J. B. Okeyo—Owuor

A survey of cowpea production and insect pests in Kenya

The cowpeas growing regions of Kenya were surveyed during the long-rain season of 1979 (26 March–2 May). The survey method involved interviews with farmers, agricultural officers, research workers and inspection of farmers' plantings and market outlets. The following information was obtained:

- The main cowpea producing areas are in Nyanza and lower parts of Eastern and Western provinces.
- Cowpeas are grown chiefly as interplants with maize and sorghum.
- The interplanting systems include mixed broadcasting, alternate rows and hill mixtures.
- Agricultural reports provide only limited information on acreage, yield and marketing of cowpeas.
- Cowpeas were marketed in most of the market places visited (approximately 50) and were produced within and outside, the district.
- The major production problems are:
  - Limited extension programme
  - Lack of "Package" production programme involving agronomic practices, well adapted varieties and pest control.
  - Limited research
- Insect Pests include the following;
  - Roots and stem pests: White grub, *Schizonycha* spp., Cutworm, *Agrotis* spp.

Leaf Pests: *Ootheca mutabilis* Shahl, *Empoasca* spp., *Aphis craccivora* Koch. *Aphis fabae* Scop., *Nezara viridula* L., *Nematocerus* spp., *Lagna villosa* F. *Epilachna* spp., Leafweb worm (unidentified), *Chilocorus* spp. *Alcidodes leucogrammus*, **Flower and pod pests:** *Heliothis armigera* Hub, Thrips (unidentified), *Maruca testulalis* Geyer. *Lampides boeticus* L. *Mirperus jaculus*, *Acanthomia* spp., *Agnoscelis pubiscens* stal. The potential for cowpeas production is high, however, little attention has been directed to agronomic practices and pest control. Further surveys are needed to provide specific information on insect occurrence and losses.

The influence of intercropping combinations on infestation

Intercropping plantings were established in replicated plots (4×4 metres) of maize and cowpeas as follows: (a) Alternate rows, (b) Hill mixture, (c) Strip cropping, (d) Inter-row mixture (e) Cowpeas alone.

The range in infestation in the various plots was:

Blossoms infested:	16.9–19.6%
Pods damaged:	30.0–46.3%
Seed damaged:	5.0–9.3%

None of the differences were significant although the lowest infestation in each category occurred in the pure cowpea planting.

It is concluded that the methodology should be modified to adequately test the influence of intercropping. Larger plots will be required.

The influence of cowpea plant type on infestation

Twelve cultivars representing three plant types were observed for levels of borer infestation. The types were:

(a) Sparse vegetative growth, moderate ground cover.

(b) Intermediate vegetative growth, moderate growth cover, semi-indeterminate.

(c) Strong vegetative growth, bushy, indeterminate.

Infestation levels were determined by counts of larvae in dropped and picked flowers, and injury to pods and seed. Data from two planting sites were inconsistent and differences were not significant.

Further refinement of experimental design will be required to determine the influence of plant type on infestation level. It is clear that the phenology of the plant and its capacity for flowering and pod set is of prime importance in considering these relationships.

Seasonal Occurrence

Counts were made of successive plantings to determine the levels of larval infestation. Collections of dropped flowers were made and the percent infested flowers determined. Observations were continued for 19 weeks, extending from the beginning of short rain season (October 1978) and continuing until the long rain season (March 1979). The study was terminated by high water which flooded the plants. There were two peaks in larval infestation coinciding roughly with heaviest rainfall. Further studies should be made to determine whether larval infestation in dropped flowers is a valid index to overall infestation of the plant.

Oviposition in relation to plant development

It is generally believed that oviposition is synchronised with flower formation. Observations were made to determine the earliest age at which plants were attractive to females for oviposition. Germination occurred on 20/8/79 and when observed three weeks later, eggs were found on 3.1% of the plants, and at four weeks at 5.0%. These findings indicate that infestation occurs before flowering and probably gives rise to adults which attack the plant at a later stage.

Host plants of the Pod Borer *M. testulalis*

It is known that the pod borer infests some wild legumes and maintains populations on them when cultivated host plants are not available. The availability of such alternate hosts undoubtedly plays an important role in the population dynamics of the pest and knowledge of these might provide options in pest management through their removal or use as trap crops.

The occurrence of the pod borer is being studied on the following: Cultivated Grain legumes, Common bean *Phaseolus vulgaris* L., Lablab *Lablab niger* L., Soybean *Glycine max* L., Pigeon Pea *Cajanus cajan* L., Ground nut. *Arachis hypogea* L., Green gram, Wild legumes, Wild pea *Vigna latiyolia, Canavalia gladiata* L.

The studies include the relative attractiveness for oviposition and larval feeding. Preliminary studies have shown that development of larvae is not significantly different on flowers of cowpea and wild pea. The pods of wildpea are not as attractive to larvae as are pods of cowpea. This may be due to the pubescence which is characteristic of the wildpea pod.

Natural enemies

Four larval parasites have been collected in the field; one Diptera and 3 Hymenoptera. Additional collections are being made in an effort to determine the natural enemies of the pest and their possible use in pest management.

Biology and behaviour

Based on field and laboratory observations the following points have been determined:

- Flight, mating and oviposition occurs at night. Flight activity begins at about 1845 hours and continues until 0630.
- Oviposition on potted plants caged in the laboratory is restricted chiefly to leaves and stems. Eggs are not deposited on flowers or green pods under these conditions. The distribution of eggs on host plants under field conditions has not been determined although eggs have frequently been observed on leaves.
- A laboratory culture has been maintained on natural food through a number of continous generations during the course of the year.
  The preferred oviposition site is along the veins of leaves. Several eggs are deposited at one site before the female moves to another location on the plant (laboratory observations).
- Mating under laboratory conditions does not occur within the first 24 hours following emergence but increases thereafter for several days.
- Females held in the laboratory and fed 5% sucrose solution varied greatly in longevity and fecundity with groups of six pairs averaging 55.4 eggs per female. Records on individual females were not obtained.

Laboratory rearing space and survival

Glass containers of varying capacity were used for rearing to the pupal stage. Twelve newly hatched larvae were introduced to each container and provided natural food. In containers having a volume of 35, 45, 70 and 300 ml. survival to pupal stage was less than 10% while in the 350ml. container survival was 37%. There was no significant difference in weight of pupae in the various tests. The factors accounting for high mortality have not been determined.

Food supply and survival

Observations were made in the laboratory on the influence of larval density on flowers on survival and pupal weight. The number of larvae per flower was 1, 2, 3 and 4. Survival was slightly higher at one larva per flower, the range being from 18% to 24% with no significant difference. There was a consistent decline in pupal weight from a high of 45 mg. for one larva per flower to 32 mg. for four larvae per flower. It is not clear whether limited food supply, crowding or other factors are responsible for this difference in size.

Physiology of aestivation–diapause in Pyralid borers

S. Yagi

Preliminary work on the physiological aspect of aestivation–diapause in the Pyralid borer, *Chilo partellus* was carried out under laboratory conditions. It is known that the larvae have aestivation-diapause in dry season and the diapause is terminated when rains reappear. Previous work at ICIPE suggested that induction, maintenance and termination of the diapause are controlled by endocrine mechanism; the diapause being regulated by juvenile hormone (HJ). However, it has not yet been possible to induce diapause by modifying artificial diets of the larvae or by treatment of non-diapausing larvae with JH. The purpose of this study was to further investigate these endocrinological relationships.

Application of JH to non-diapausing larvae

Larvae were reared continuously on an artificial diet in the ICIPE Insectary. JH analogue (JHA, Zoecon ZR-515) was added to the diet of 4–5th instar larvae at 12.5–125ppm 2 or 3 times per week to examine the effects of the hormone. No larvae treated with JHA pupated within 2 months after treatment although almost all non-treated larvae pupated. In the case of control (acetone), few larvae pupated but the majority survived as full grown larvae.

From these results it is suggested that JH may regulate the induction and maintenance of diapause of this borer. However, further experiments will be needed to clarify the factors regulating diapause.

Effects of beta-ecdysone on field collected larvae

It is important to determine diapause intensity of the larvae in the field for forecasting borer infestation. Thus, *Chilo* larvae (last instar) obtained from sorghum stems collected from Mbita Point Field Station were individually injected with 4μg of beta-ecdysone every month as was previously done in the case of the rice stem borer, *Chilo suppressalis*. Results are shown in Table 7. All of the field collected larvae ecdysed to larvae or larval-pupal intermediates. On the other hand, all of the non-diapausing last instar larvae of *C. partellus* obtained from the ICIPE Insectary ecdysed to pupae or more advanced larval-pupal intermediates after receiving the hormone.

Table 7. Effects of beta-ecdysone on last instar larvae of *Chilo* sp. from Insectary and Mbita Point*

Source	No. of larvae used	No. of larvae unaffected	No. of larvae ecdysed to: Larvae	Intermediates** +	Intermediates** ++	Pupae
Non-diapausing larvae, Insectary	24	0	0	0	8	16
Larvae from Mbita Point (October, 1979)	14	0(4)***	8	1	1	0

* Each larva was injected 4 μg of beta-ecdysone and observed for one week.
** (+) slightly advanced and (++) more advanced larval -pupal intermediates.
*** Number in parenthesis shows the number of larvae which died before ecdysis.

From these preliminary results, it is suggested that it may be possible to reveal the change of JH titer during the aestivation-diapause. In other words, diapause intensity of the larvae may be revealed by injecting beta-ecdysone into the larvae in various stages; this is because during diapause all treated larvae underwent larval-larval or larval-pupal intermediate ecdysis, while pupae were obtained from treated non-diapausing larvae.

Oviposition Site Selection Behaviour of the Sorghum Shootfly *Atherigona soccata*

K. Ogwaro

It is recognized that non-preference for oviposition is a major mechanism of resistance to the shootfly. To better understand this phenomenon, observations

were made under laboratory conditions and the distribution of eggs under field conditions was also studied. Some of the findings are:

- Time spent in searching for oviposition sites can be used as a criteria for preference.
- The presence on a leaf of eggs deposited by the female or deposited by other females does not preclude further oviposition under laboratory conditions in which oviposition sites are limited. These results appear to be at variance with oviposition pattern under natural conditions.
- The preferred oviposition site is the lower side of the leaf. This preference is apparently not due to morphological differences in the two sides but is more likely a positional factor or one of light intensity (shade).
- The preferred leaf for oviposition was number III and this preference is unchanged from germination to 30 days later.
- In mixed age plantings, the preferred size for oviposition was within the range 30–35 cm. in height.
- Leaves in the range of 25–30 cm. in length are the most attractive for oviposition.
- Plants are most attractive to oviposition at 36 days after germination with a sharp decline in attractiveness thereafter.
- The females show marked preference for *Sorghum bicolor* over other hosts tested (*Setaria verticillata, Digitaria scallarum, Panicum maximum*).

Effect of Maize, Cowpea and Sorghum intercropping combinations on infestation by some insect pests

E. M. Omolo

Intercropping is the traditional practice of subsistence farmers of the tropics. While it is generally recognized that this system which evolved over extended periods of trial and error provides some advantages, little research has been directed to the resulting ecological relationships and the ensuing possibilities for pest management.

Studies were undertaken to determine infestation levels of sorghum shootfly *Atherigona soccata* and stem borers (*Busseola, Chilo* and *Sesamia*) under single crop and intercropping combinations as follows: maize, sorghum, cowpea, maize/sorghum, maize/cowpea, maize/sorghum/cowpea.

Fewer eggs of shootfly were deposited on young sorghum plants in the maize/sorghum combination but these relationships did not apply as the plants reached the tillering stage (sorghum). Combinations of maize/sorghum were more severely attacked by borers than maize/cowpea.

The data were not conclusive suggesting that plot design and methodology were not well suited to the objectives of the test. This preliminary test indicated the added complexity of research on multiple cropping as compared to monoculture when determining levels of insect injury and effect on yields. Clearly, a new orientation and methodology is required as a prerequisite to progress in mixed cropping entomology.

Larval Instars of the Pod Borer *Maruca testulalis*

J. Bayo Odebiyi*

The head capsule widths of larvae of *M. testulalis* reared individually and in groups in the laboratory were determined. The measurements were subjected to analysis of variance, frequency distribution and Dyar's rule. The results are given in Table 8.

Table 8. Head capsule measurements of larval instars of *M. testulalis*

Instar	Range (mm)	N	Mean ± S.D. (mm)	Increase (mm)	Growth Rate	Length of Stadium
						(Days)
I	0.195	30	0.195±0.0	—	—	2
II	0.312—0.351	27	0.338±0.019	0.14	1.73	2
III	0.585—0.624	25	0.594±0.017	0.26	1.76	1—2
IV	0.897—1a014	16	0.957±0.035	0.36	1.61	1—2
V	1.326—1.442	41	1.381±0.036	0.42	1.44	3

(N — sample size).

* Research Associate Scientist from Entomology Unit, Department of Agricultural Biology, University of Ibadan, Ibadan, Nigeria.

The distribution pattern, of the head capsule widths is distinctly unimodal and separable into five nonoverlapping groups. Variation in length of the different instars, although small, resulted in an overlapping of the larval instars especially in the last two. The determination of larval instars provides a useful point of reference for further studies on larval biology and ology.

Visiting Directors of Research
- Professor W. S. Bowers (1977)
- Professor R. Galun (1970)
- Professor J. H. Law (1977)
- Professor M. Locke (1977)

Programme Leader
- Dr. M. P. Cunningham (1977)

Research Staff
- Mr. A. Bwire (1975) Technical Assistant
- Mr. J. W. Chiera (1976) Research Assistant
- Dr. G. M. Binta (1978) Associate Scientific Officer
- Mr. G. M. Hindi (1974) Subordinate Assistant
- Mrs. C. K. A. Mango (1971) Scientific Officer
- Mr. A. O. Mongi (1975) Graduate Trainee
- Mr. J. G. Mugane (1973) Subordinate Assistant
- Mr. J. N. Ndungu (1973) Subordinate Assistant
- Dr. R. M. Newson (1974) Senior Research Scientist
- Dr. F. D. Obenchain (1976) Research Scientist
- Mr. R. Ojowa (1972) Junior Technical Assistant
- Mr. D. K. Punyua (1973) Associate Scientific Officer
- Mr. F. T. Thuo (1977) Technical Assistant
- Mr. K. C. Wainaina (1974) Subordinate Assistant

Collaborators
- Dr. K. Cowan, USDA, KARI*
- Dr. T. T. Dolan, KARI
- Dr. B. J. Ellis, Georgia Southern College, USA
- Dr. A. D. Irvin, ILRAD**
- Mr. B. L. Leitch, KARI
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- Mr. L. Moreka, Bioassay
- Mr. E. N. Ole Sitayo, Bioassay
- Dr. D. Otieno, Chemistry/Biochemistry
- Dr. S. Waladde, Sensory Physiology
- Dr. D. Whitehead, Chemistry/Biochemistry
- Dr. A. S. Young, KARI

*Kenya Agricultural Research Institute

**International Laboratory for Reasearch on Animal Diseases

ECOLOGY

Introduction

During 1978 we realised that natural development of resistance to tick infestation by host cattle could have profoundly influenced the numbers of *Rhipicephalus appendiculatus* in previous field experiments. We are now, therefore, investigating some of the effects of this resistance on ticks at both the individual and population levels. In parallel with this we have begun some intensive studies of tick behaviour on the host and survival on the ground.

Development of *Rhipicephalus appendiculatus* populations at different stocking densities

R. M. Newson and J. W. Chiera

This study was begun in June 1976; the final cattle observations were made in April 1979, but some ground collections were continued throughout the year. Five cattle with little or no previous experience of *R. appendiculatus* infestation were placed individually in fenced plots (nos. 1 and 3, 1,000m^2; nos. 4 and 5, 4,000m^2; no. 6, 12,000m^2). The plots were infested artificially with unfed *R. appendiculatus* larvae (9/m^2) and nymphs (4/m^2). Thereafter the tick numbers were closely monitored whilst feeding on the host (adults) and when waiting unfed on the vegetation to be picked up by the host (mainly larvae and nymphs).

The tick populations on all plots increased rapidly, reaching their highest numbers of females per host in early 1977 (examples given in Figure 1). All five populations behaved comparably; only the actual levels of numbers differed somewhat, and this was confirmed by the ground collection data for larvae and nymphs. Thus there were well-defined cohorts of adults, larvae, nymphs then adults again.

The most rapid and pronounced changes in numbers occurred on the smallest plots where the mean waiting time until pick-up by the host must have been shorter, and also the survival rate during this period must also have been poorest due to heavy grazing by the same hosts on the protective grass cover. By contrast, the biggest plot had the best conditions for survival during the large proportion of the lifespan that is spent off the host, which was counter-balanced, however, by the twelve times lower chance of a tick feeding in any given period. The result was that in plot 6 the tick population oscillated much more gently then elsewhere (Figure 1).

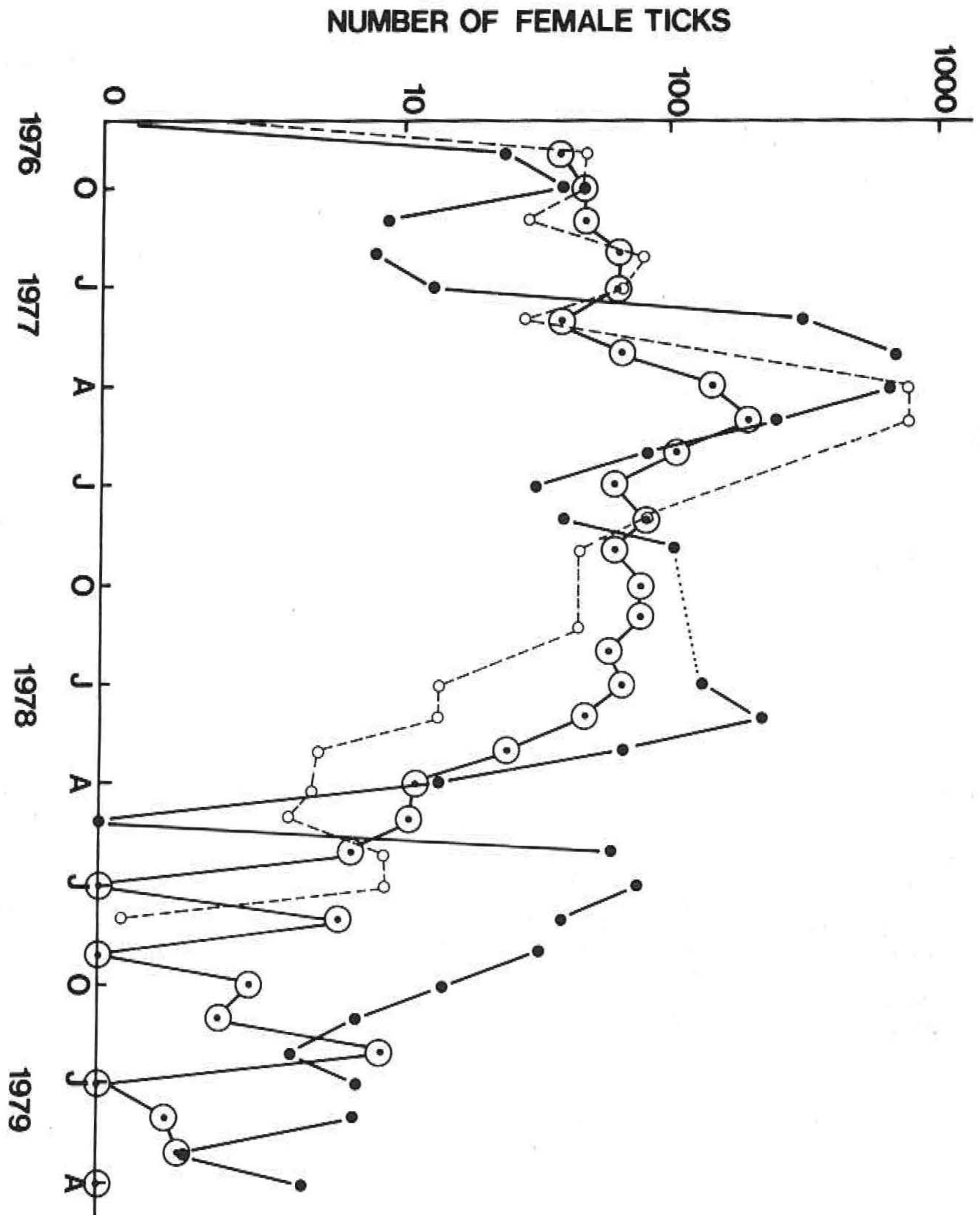


Figure 1. Total numbers of female *R. appendiculatus* attached to the host, estimated from monthly half body collections; plot 3 ● —— ●; plot 5 – – – –; plot 6 ⊙ ⊙. Plot 3 was destocked September – December 1977.

Nevertheless, after this first exuberance all the adult tick populations began to decrease, although large numbers of larvae and nymphs were also still being detected on the ground. Previous observations have shown that *R. appendiculatus* adults are most abundant on cattle during the long rains, with a sharp increase in numbers when the rains begin in April each year. Renewed adult activity might have been expected, therefore, in the corresponding periods in 1978 and 1979. This did not occur, and by April 1979, the *R. appendiculatus* populations on all five plots were close to extinction.

It now seems that host resistance to tick infestation developed during the phase of rapidly increasing numbers and, as will be demonstrated below, this reduced survival during feeding (especially of the larvae), as well as impairing reproductive performance in the females. Thus a vicious circle was initiated in which adults continued to be picked up from the ground for a further two years from the existing stock, though with ever-decreasing benefit to the tick populations. At the same time the high population of immatures produced in the latter part of 1977 met with little success in its attempts to feed, and in the absence of adequate replacements from the breeding activities of the adults, it died out during 1978, followed inevitably by the adult population a few months later.

The effect on *R. appendiculatus* of feeding on tick-resistant hosts

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

Large numbers of adults, nymphs and larvae were fed on pairs of *Bos taurus* calves and control rabbits which had never fed ticks before, in order to establish base-line data on percentage engorging, days to engorgement, engorged weight and percentage subsequently laying eggs or moulting (Table 1). Samples were also taken on each day of feeding and the individual ticks weighed and measured. We also showed that there was little change in these parameters when successive batches of 100 nymphs were fed on two initially tick-naive cattle. Single test feeds of 100 nymphs can therefore be safely used to determine the resistance status of cattle to tick infestation. It is now being used by us routinely. The test was then applied to the five cattle used in the stocking density experiment immediately after their removal from the paddocks, in addition to test feeds of 100 larvae and 20 adults (Table 2). A degree of resistance was indicated that could largely explain the population declines that were observed.

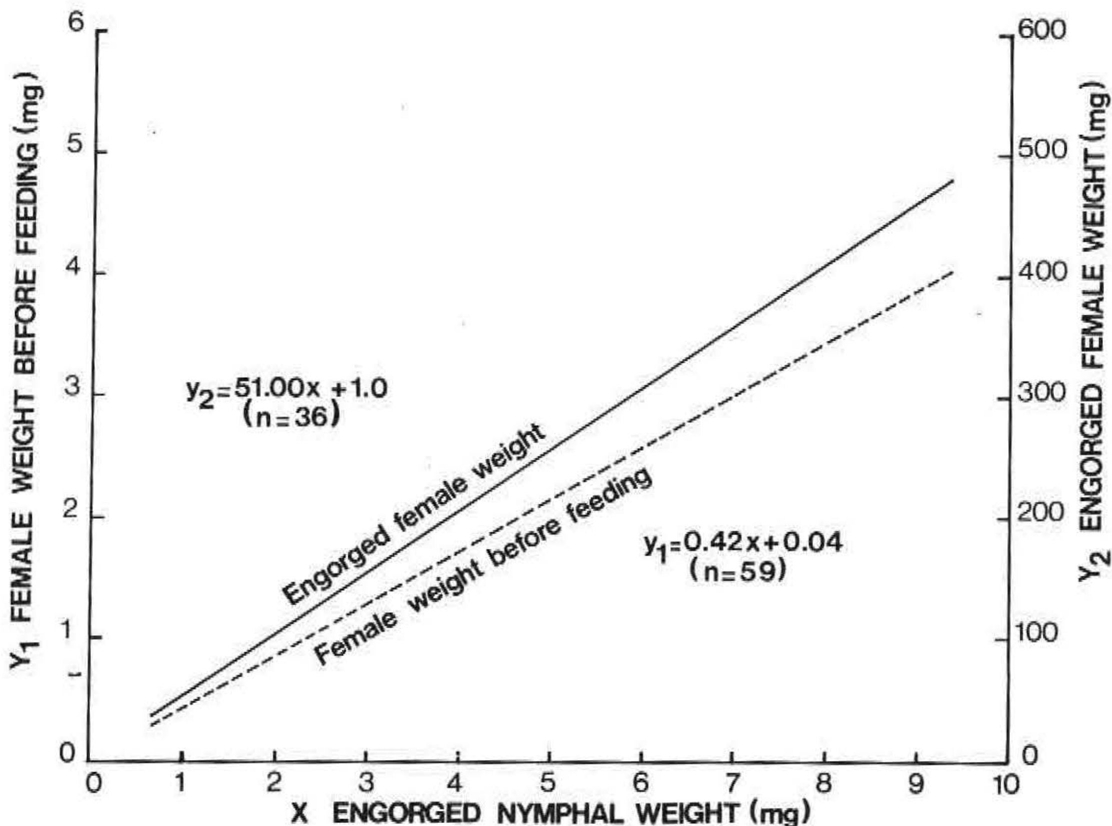


Figure 2. Relationship between engorged weight of the nymph of *R. appendiculatus* and the weight of the unfed female and the engorged female (fed on a susceptible host).

Finally a series of results was obtained on engorged nymphal weights, unfed adult weights and engorged female weights showing that these are all closely correlated (Figure 2). For females the engorged weight (and thus the number) of eggs laid is also correlated with the unfed weight.

The effect of host resistance on tick population development

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

Three double-fenced paddocks of approximately 0.6 ha each have been prepared at the Kenya Agricultural Research Institute, Veterinary Research Department, Muguga. Each was seeded with unfed larvae of *R. appendiculatus* at a mean density of $21/m^2$. Two resistant cattle (from previous field experiments, identified by the test described above) were put into one paddock. Two tick-susceptible cattle were put into each of the other paddocks. The hypotheses being tested are that:

(a) There will be little or no production of nymphs or adults in the presence of resistant hosts, but there will be good yields in the other two paddocks.

(b) The breeding success of any adults reared on resistant cattle will also be lower than that of ticks reared on susceptible cattle.

(c) The susceptible cattle if allowed to remain in one paddock will develop resistance as described above.

(d) If initially susceptible hosts are changed for fresh susceptible hosts every three months the increase in tick numbers will be rapid and sustained.

Ticks are being monitored as before, with additional samples of larvae and nymphs obtained by scraping all the ticks from small selected areas of skin.

The cattle exposure began in October. One of the resistant hosts showed a strong immediate hypersensitivity reaction to the larvae three days later, with virtually none feeding successfully. However, by the eleventh day the reaction had subsided and the number of feeding larvae was similar to all the other hosts, which was only 40% of that on day 3. It was estimated that by this time a minimum of one third of the introduced larvae had already been picked up by the cattle.

Field assessment of resistance to tick infestation and its development in calves

R. M. Newson and D. K. Punyua

A study has been started at Lolgorien in Narok District, in collaboration with the staff of KARI, Veterinary Research Department, who are making a detailed field study of calf health and growth in selected herds. Our objectives are to provide background information on tick infestation for correlation with their results on tick-borne diseases, as well as investigating resistance to tick infestation in the observed herds. The cattle are chronically infested with *R. appendiculatus* and at least five other tick species. We shall follow the development of resistance from calfhood to adulthood and try to assess its influence on the population dynamics of *R. appendiculatus*.

The results of the first three visits to the area indicate that adult tick infestations are markedly higher on the cows than on the calves, but feeding success has yet to be measured. The unfed ticks themselves average 16% smaller than the Muguga colony strain and we are at present culturing ticks from Lolgorien to see if this difference is phenotypic or genotypic

Table 1. Mean feeding performance of *R. appendiculatus* on pairs of tick-naive calves, and pairs of naive control rabbits; the percentage moulting or laying eggs is based on the number engorging

Feeding site	Days to engorge	Engorged weight (mg)	% engorging	% moulting or laying	No. applied per host
		Females			
Calf ears	6.5	364.7	91		100
Calf body	6.5	352.2	95	99	100
Rabbit ears	8.1	378.6	98	98	25
		Nymphs			
Calf ears	6.2	9.11	81	81	500
Calf body	4.8	9.65	79	98	500
Rabbit ears	5.6	8.40	86	95	250
		Larvae			
Calf ears	3.8	0.39	60	85	1,000
Rabbit ears	4.8	0.51	86	97	500

Table 2. Mean feeding performance of *R. appendiculatus* on cattle showing resistance to tick infestation at the end of the stocking density experiment, compared with naive rabbit controls. The adults were fed first followed immediately by the immatures

Feeding site	Days to engorge	Engorged weight (mg)	% engorging	% moulting or laying	No. applied per host
		Females			
5 cattle (l. ear)	6.8	253.1	56	86	10
2 rabbits (l. ear)	8.5	382.1	85	100	10
		Nymphs			
5 cattle (r. ear)	4.2	3.71	15	80	100
2 rabbits (r. ear)	7.0	8.56	98	99	100
		Larvae			
5 cattle (l. ear)	3.5	—	< 1	0	100
2 rabbits (r. ear)	4.0	0.48	58	85	100

Survival of *R. appendiculatus* ticks in the field

Daniel K. Punyua

This long term experiment is designed to obtain survival patterns, activity and viability of ticks kept in the field until the last viable individual has been recovered. Known numbers of ticks at different stages of development, just at the point of moulting (larvae and nymphs) or hatching (eggs), are separated in the laboratory. Some of the ticks are released into circular plots (1 m. diameter) surrounded by 30 cm high walls made of plain iron sheets. Another group of ticks is confined in nylon mesh tubes (30cm × 2cm) sealed at both ends and the side sewn with nylon thread. Another group of ticks is confined in 4cm × 3cm nylon mesh bags prepared as above.

During release, the nylon mesh tubes containing ticks are placed vertically in the vegetation, and the bags horizontally at the soil level. Sampling is carried out by hand picking the adults and flagging the plots containing the immature stages.

A number of the plots containing each of the instars is sampled once every week, others once every month and the last group once every four months. All the live ticks are counted and transferred to rabbit ears for viability testing and feeding performance.

The experiment has been going on for three months and it is too early to judge the results. One important observation is that the immatures become active immediately on release. From the weekly sampled plots an average of 63% of the total number of larvae released (range 40-78%) and 64% of the released nymphs (range 58-67%) were recovered. The adult weekly samples have yielded a mean of 4% and 11% of the *in situ* counts and hand picking respectively.

As against the weekly samples the monthly samples have yielded 0.7% of the adults, 24% of the nymphs and 33% of the larvae.

The feeding performance of the field ticks compared with the laboratory strain seems to be the same in all the stages.

Behaviour of the male *Rhipicephalus appendiculatus* on the rabbit host

D. K. Punyua

Introduction

In adult tick populations the only reliable index for assessment is by counting the adults on the hosts. *R. appendiculatus* males are known to remain on their hosts much longer than females, but their overall effect on population changes and their role in disease transmission has not been investigated. For this reason an experiment was designed to study the behaviour of *R. appendiculatus* males on rabbit hosts.

Using different colours of enamel paint, six months old males and females were each numbered by application of small spots of paint on the scutum of each tick with the tip of a needle. As soon as the paint was dry ten marked ticks of each sex were applied to each rabbit ear. Two days later the position of each attached individual male or female was noted on a map of the ear. Daily, thereafter, the position of each tick was noted on the map taking into account whether the tick was *in copula* with a female or not. Fertilization was judged to have occurred when a female seen *in copula* with a male and subsequently engorged, laid eggs, and the eggs hatched. Introduction of freshly numbered females into the rabbit ear was done as soon as the female of the previous group had dropped off. This continued until the males were either unable to copulate or were dead.

After a number of female applications the rabbit developed some degrees of resistance and only very few or none of the applied females managed to feed to full engorgement, despite the fact that most of them

were mated and some laid eggs which subsequently hatched into viable larvae. This unforseen development undoubtedly complicated the interpretation of the results. From Table 3 it can be seen that although a number of matings took place, some of these were unsuccessful in that the females either failed to lay eggs or the laid eggs failed to hatch. It was not possible, therefore, to attribute this behaviour to either the host resistance or to male exhaustion. The longest surviving males remained on the host for 76 days. Twenty matings were recorded for one individual male, out of which only 13 matings were successful.

One other finding is the degree of male movement on the host. Male ticks detach and reattach 2-3 times daily. The reason for this phenomenon is not clear.

Although not a very common phenomenon, repeated mating, either by the same male with the same female or with a different female was occasionally observed.

Table 3. Behaviour of *R. appendiculatus* males on the host

Male number	Days surviving on host	No. of migrations	No. of unsuccessful matings	No. of successful matings	No. of repeated matings	Total matings
1	76	26	3	6	1	10
2	34	10	1	4	0	5
3	61	29	4	13	3	20
4	76	25	3	6	0	9
5	36	12	1	5	0	6
6	76	31	3	8	1	12
7	57	21	1	9	1	11
8	44	21	1	12	0	13
9	58	23	1	8	2	11
10	45	19	1	4	0	5
11	10	3	1	1	0	2
12	65	21	3	10	1	14
13	34	12	1	6	0	7
14	6	3	0	1	0	1
15	—	—	—	—	—	—
16	25	10	0	6	0	6
17	40	17	1	7	0	8
18	45	20	2	6	0	8
19	10	3	0	1	0	1
20	22	10	0	6	0	6

PHYSIOLOGY

A. Studies on Tick Pheromones and Behaviour

Amblyomma aggregation-attachment pheromones; the behaviour of *A. cohaerens* and general observations on species specificity.

F. D. Obenchain, R. Newson, R. Ojowa and F. Thuo

Observations reported in previous numbers of the ICIPE annual report have confirmed the existence of a pheromonal mechanism which controls the attachment behaviour of female ticks of a number of Kenyan species of *Amblyomma* ticks (*A. variegatum, A. gemma and A. eburneum*) and its absence in another species (*A. falsomarmoreum*, a parasite of leopard tortoises). When the mechanism is present in a species, females show a characteristic reluctance to attach to available hosts in the absence of fed males (10 days) which have matured sperm and are therefore ready to mate. Based on observations within scrotal bags or stockenette arenas between the inter and intraspecific attachment responses of the three species which exhibit the aggregation pheromone mechanism, it appears that the pheromone must only be active over short distances; original contact between the sexes is primarily due to the general exploring activity of the females. Moreover, the degree of species-specificity of the pheromonal mechanism seemed to be variable. Females of *A. variegatum* and *A. gemma* did not seem to discriminate between pheromone producing males of their own or the other species. Females of *A. eburneum*, however, would not aggregate or attach to rabbit hosts in the presence of pheromone producing males of *A. variegatum*. These data suggested that there might be a spectrum of pheromone speci-

ficities among the various species of *Amblyomma* ticks.

Observations on the above mentioned species of *Amblyomma* also showed that there was a marked difference in the attachment times of a group of females when they were placed in a bioassay arena with a single pheromone producing male; the first female to contact the male rapidly attached to the rabbit host in a venter-to-venter position with the male. The other females in the assay arena attached much later. For that reason the assay was not scored until 24 hours after introduction of the females.

In mid-1979 an ecological survey of the cattle-tick interactions in Lolgorien showed that two species of *Amblyomma* were present on cattle. The more common species was *A. variegatum*, but males and females of *A. cohaerens* were also feeding on the cattle. This latter species is reported to be common on cape buffalo which abound in the area. Both species of adult *Amblyomma* were found on the ground in the enclosures where the adult cattle are kept at night. Because of the overlapping habitats and distribution of ticks on the cattle (both species feed on the *lower body surfaces and heels*) it seemed reasonable to determine whether *A. cohaerens* ticks exhibited the aggregation-attachment pheromone mechanism and, if they did, to investigate the species specificity of the mechanisms.

The bioassays for pheromonal activity on the part of 10-day fed males were performed on rabbits as described in previous annual reports. These tests showed that females would not attach in the absence of a fed male *A. cohaerens*. Both sexes, however, were reluctant to attach to rabbits. This supports the observation that adult *A. cohaerens* have a marked host preference for buffalo. When 5 females were placed with a fed male *A. cohaerens* in a series of 4 replicates the characteristic response was for one female to attach rapidly and for the rest of the females to be unattached after 24 hours. Female *A. cohaerans* did not attach in the absence of a fed male of their own species; they apparently do not recognize the pheromone produced by fed male *A. variegatum*. In this situation of marked host non-preference, the role of the male in inducing attachment appears to be two-fold. Sexually mature males of both *A. cohaerens* and *A. variegatum* respond quickly to contact with an exploring female; first, they raise their bodies into a vertical position in relationship to the host and, second, they actively grasp the female with their legs. When the male grasps the female she appears to be exposed more directly to the males pheromone. When a female *A. cohaerens* is grasped by a male *A. variegatum* she appears to become less active for a short period of time, but she resumes her activity and leaves the male without attaching to the host. By contrast, when grasped by a male *A. cohaerens* the female remains less active for some time. If the male has grasped the female in an unfavourable position for her attachment, he may then rotate her until her mouthparts are orientated towards the host, following which she attaches to the host. When other females contact the occupied male he shows little reaction and the female does not attach. It appears that in this situation where the rabbit host is not preferred, an active intervention on the part of the male is needed in order to properly expose the female to the source of the "aggregation-attachment" pheromone. In such a case, attachment is induced on a one-to-one basis between males and females and aggregation is not observed to the extent previously reported for *A. variegatum*, *A. gemma* and *A. eburneum*. Before these observations can be extended to a general interpretation of the pheromonal mechanism of other *Amblyomma* species it might be necessary to study the attachment behaviour of *A. cohaerens* on their preferred host, the cape buffalo.

Argasid assembly pheromones; active components from the nitrogenous excreta of *Ornithodoros porcinus porcinus*.

F. D. Obenchain, D. Otieno and A. Bwire

Previous work at ICIPE showed that various species of soft ticks (the family Argasidae) assembled off the host in response to some chemically active "exosecretion". This material was usually collected by washing the ticks in saline or by collecting pieces of filter paper which had been exposed to recently fed ticks. Papers with high activity were usually those containing large amounts of nitrogenous excreta. In order to determine if the excreta was the source of the active component, material was collected from several hundred virgin female *O. p. porcinus* ticks. Since the passage between the mid-and hindgut is permanently blocked in all stages of this tick there was no possibility that the nitrogenous excreta (known to be principally guanine) could be contaminated with intermediate products of the digestion of the bloodmeal (such as hematin, etc.) The nitrogenous excreta of these female *O. p. porcinus* were disolved in various solvents and active washes (by bioassay) were separated by HPLC as described in the Chemistry section of this annual report. The fractions obtained in that way were then bioassayed for assembly pheromone activity as reported previously. Nymphal *Argas persicus* were used as the test ticks. The bioassay arena was a 5cm petri dish (uncovered) which was placed on a heating table at 32°C. Six small filter paper disks (1.5cm) were placed in the petri dish in an hexagonal pattern. One disk was treated with the test fraction of nitrogenous excreta and the remaining 5 were treated with the solvent. Ten ticks were placed in the center of each petri-dish-arena, the dishes were covered with an opaque cover and the ticks were left in darkness for 3 to 24 hours. After the test period the cover was removed and the distribution of the ticks (all were in contact with one or another disk) was scored.

A characteristic chromatogram of the tick nitrogenous excreta (see Chemistry section of this report) showed 5 peaks. In 4 replicate bioassays of the 1st peak the ticks showed no assembly on the treated disk, but were found on the average more often on the control treated disks (0.6 to 1.0). When material collected from the trailing shoulder of peak number 1 was bioassayed it seemed to show some significant repellancy. No ticks were found on the treated disk while the untreated disks showed an even distribution of the test ticks. The first peak probably consists of sodium and/or potassium chloride. The second and third peaks were not always resolved in the chromatograms and their fractions were pooled for bioassay. In 4 replicate trials, the ticks were more often found on the treated disk than on the control disks (2. 9 to 1.0). These peaks have not been chemically identified at the time of this report. The greatest assembly pheromone activity was associated with the 4th peak which was identified as guanine. Ticks were found on disks treated with the guanine fraction in a ratio of 9.1 to 1.0 by comparison with the solvent-control treated disks. When chemically purified guanine from a number of commercial sources was tested it was also found to be active. The fifth and final peak has been identified as adenine. It was present in such low concentrations that it was not feasible to collect the fraction for bioassay. Commercially available adenine has not yet been tested for activity.

Although guanine had previously been known as the major (approximately 90%) nitrogenous excretory product of ticks, there had been little speculation on its possible role as a pheromone. It is also known that some ticks have a second purine component in their excreta which may account for approximately 5% of total wastes. It seems probable that this second purine is present as peak number 2 or 3. The question then arises as to the specificity of the ticks sensory mechanisms. Are they sensitive to only a few purines which are present in their own wastes or will they react to other purines or an even broader range of nitrogenous compounds. The answer to this question must be determined before any further attention can be given to the use of argasid assembly pheromones in tick control. Argasid ticks are usually nest, burrow or cave inhabitants. In such a habitat they would also be exposed to the nitrogenous wastes of their vertebrate hosts which would be present in amounts several orders of magnitude greater than the ticks own wastes. Tick assembly in response to guanine baited with acaricide would occur only if the tick does not respond to the nitrogenous excretory components of the host.

Engorgement rates among female *Rhipicephalus appendiculatus:* effect of the presence or absence of sexually mature males.

F. D. Obenchain, S. Waladde and R. Ojowa.

In order to test for the significance of the effects of the presence of sexually mature males on the engorgement rates of recently attached *R. appendiculatus* females the following experiment was performed. Six groups of 35 unfed *R. appendiculatus* females were generated by random selection techniques from a population of approximately 600 females obtained from the ICIPE-KARI tick strain which is maintained at Muguga. Each group was placed in an ear bag on one of 3 litter-mate laboratory rabbits (6 months old) none of which had been used previously as a tick host. Five-day fed males (70) were already attached and feeding on the right-hand ear of each rabbit. Two days after female attachment all ticks were removed from the 1st rabbit. Females were weighed immediately and placed in individual vials for later determinations of their scutal measurements. All ticks were removed, weighed and measured from rabbits 2 and 3 on the 3rd and 4th days post-attachment, respectively. After measurements were made, female ticks were individually dissected to confirm that mating had not taken place. In fact, none of the females had been mated by the time feeding was terminated on day 4 post-attachment.

Relative engorgement state (RES) ratios were computed for all ticks and the ratios converted to logs for analysis. It had been previously determined that log RES data for consecutive days were approximately normally distributed, with equal group variances. Table 4 summarizes the two-way analysis of variance for the log transformed data. The computed F statistics for differences among the group means for days of feeding and for treatments (presence or absence of sexually mature males), as well as for the interaction between these factors are all very highly significant. In the subsequent one-way analyses of variance for successive days of feeding (Table 4) it can be seen there was no difference between the group means on day 2 of feeding. Females feeding alone had a mean log RES value of .0439 compared to .0445 for females feeding with males present. By the third day of feeding the mean log RES for females alone was .0813 and for females feeding with males it was .1131. These means were statistically different at the .01 confidence level. The differences on the 4th day of feeding were significant at the .001 level; females feeding alone had a mean log RES of .1589 while females feeding with males had a mean of .2710.

Table 4. Analysis of differences in the engorgement states (log RES) during the 2nd, 3rd and 4th days of feeding by *R. appendiculatus* females in presence and absence of sexually mature males

I. TWO WAY ANALYSIS OF VARIANCE: Factors influencing the differences among means.

Hypothesis Tested	df	F	P
Differences among days	2 & 204	272.106	.001
Differences among treatments	1 & 204	61.995	.001
Differences due to interaction of days and treatments	2 & 204	29.746	.001

II. ONE WAY ANALYSIS OF VARIANCE: Tests of significance of differences between daily treatment means

Hypothesis Tested	df	F	P
Differences on day 2	1 & 68	0.023	NS
Differences on day 3	1 & 68	7.594	.01
Differences on day 4	1 & 68	67.371	.001

III. COMPARISONS OF DAILY TREATMENT MEANS:

	Females, but no males		*Females plus males*	
	mean log RES	coefficient of weight increase	mean log RES	coefficient of weight increase
Day 2	.0439	1.35	.0445	1.36
Day 3	.0813	1 75	.1131	2.18
Day 4	.1589	2.99	.2710	6.50

The comparisons can be made in simpler terms as follows: females feeding in the absence of males had increased their starting unfed weights by 1.4, 1.8 and 3.0 times on days 2, 3 and 4 post-attachment, respectively, while females feeding in the presence of sexually mature males had increased their weights by 1.4, 2.2 and 6.5 times in the same time periods.

While these data clearly establish the importance of male presence on the promotion of early female engorgement, the mechanisms involved are unknown. However, there appears to be some evolutionary sense to the existence of such a mechanism. Females arriving on a host before males would feed slowly and be less susceptible to predation by tick birds or to the grooming activities of the host. The female would then increase her feeding rate as males began to feed nearby and would shift into the rapid last phase of engorgement when mated. Females arriving on a host which already supported sexually mature males would feed more rapidly during the early phases of engorgement, would mate earlier and would drop from the host in the minmum possible time. This mechanism would tend to reduce the relative frequency of the conspicuous partially engorged female stages on a host. In a situation of low tick density and high predation or grooming activity this type of behaviour could have a very significant survival value.

B. Studies on tick endocrinology

The effects of beta-ecdysone and ponasterone A on the soft tick *Ornithodoros porcinus porcinus* Walten, 1962.

C. K. A. Mango

The lack of basic information in the field of tick endocrinology led to this investigation on the effects of an insect moulting hormone, beta-ecdysone, and a phytoecdysone, ponasterone A, on adult and nymphal *O. p. porcinus* ticks.

Ticks were bred at 28°C with a relative humidity of 75 to 80% on four laboratory hosts: rabbits, chicken, rat and guinea pig. *In vitro* breeding techniques were also used to study bovine and porcine defibrinated blood as possible substrates for the mass rearing of ticks. The suitability of a host or substrate was assessed by the feeding performance of the ticks, their reproductive efficiency, the percent hatch of eggs and subsequent moulting success and rate of adult maturation. Observations indicated that rabbit-and chicken-fed ticks laid more eggs per unit weight of bloodmeal and the resulting eggs had better hatching rates than rat-and guinea pig-fed ticks. The moulting success and rates of development and adult maturation were also better among rabbit-and chicken-fed ticks than rat-or guinea pig-fed ticks. Porcine blood was an excellent substrate for the promotion of high levels of egg output while bovine blood gave poor results. In some respects, rabbit blood was a superior substrate in comparison to porcine blood but rabbit blood haemolyses easily and it is not suitable because of resulting tick deaths. Porcine blood was greatly superior to bovine blood in most parameters of tick feeding and development.

Topical application of beta-ecdysone (1 to 5μg/tick) to adult *O. p. porcinus* gave higher mortality among males than females, while both males and females supermoulted. Topical application of beta-ecdysone and ponasterone A to 2nd through 5th nymphal instar ticks, the same day or 4 days post-feeding, produced higher mortality among treated nymphs but the pre moulting period was also shortened among nymphs treated 4 days post-feeding. There were differences in the rates of moult acceleration among the surviving nymphs with different ecdysteroid doses and in some cases the dose responses were linear.

Ticks fed on bloodmeal with added ecdysteroids at 1 to 8μg/ml of blood showed low (5.2%) and high (41.3%) mortality responses among female and male ticks, respectively. Among ponasterone A treated ticks, a high frequency of mortality ranging from 3.2 to 90%, was observed among both male and female ticks. Supermoulting among beta-ecdysone fed ticks occurred at higher (74%) and lower (19.3%) rates among females and males, respectively. Ponasterone A fed ticks also showed higher (55.8%) rates among females than among

males (21.6%). Some beta-ecdysone fed females underwent a second supermoult on the ecdysone from the first bloodmeal and the dose response curve for this response was linear. Among nymphal instars, feeding on ecdysteroids caused moult acceleration, the rate of which depended on the nymphal stage. Older (and larger) nymphal stages experienced better moult acceleration than the younger (and smaller) ones. Age did not affect supermoulting potential in *O. p. porcinus*. Both *A. persicus* and *O. tholozani* showed a very slight supermoulting response when fed on bloodmeal with added ecdysteroids.

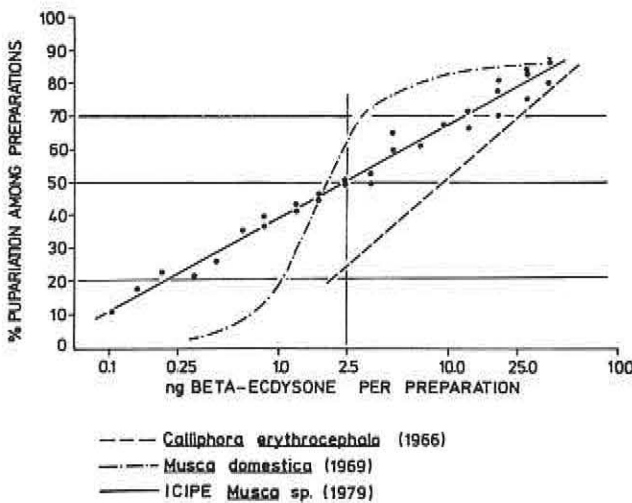


Figure 3. Comparison of the dose-response curve in the ICIPE *Musca* bioassay for beta-ecdysone activity to the *Calliphora* bioassay of Karlson (1966) and the *Musca domestica* bioassay of Adelung and Karlson (1969).

Figure 3 shows the dose response curve of an ICIPE strain of *Musca sp.* larvae to various doses of injected beta-ecdysone standard which was used in the calculation of the results. Moulting hormone equivalent titre changes (determined by *Musca* bioassay) in the haemolymph of normally-fed 5th instar nymphs occurred in a pattern with 2 peaks; the lower 1st peak appeared on day 4 and the higher 2nd peak appeared on day 9, just before moulting. Ecdysone-fed 5th instar nymphs showed an early titre peak on day one followed by a normal pattern with the 2 peaks. Normally-fed and mated females showed a low titre peak on the 7th day post-feeding which coincided with the onset of vitellogenesis. In ecdysone-fed unmated females, the 2 basic peaks of nymphal moulting pattern were observed; an early lower moulting peak appeared on day 5 and a 2nd higher peak appeared on day 10, preceding supermoulting. In the ecdysone-fed mated females, the 2 basic titre peaks of nymphal moulting pattern also occurred. Comparisons between the equivalent moulting hormone titre changes in normally-fed 5th instar nymphs and ecdysone-fed females were essentially the same, both in terms of timing and in the magnitude of the changes.

The biology of the supermoulted ticks revealed that while the supermoulted ticks grew larger with subsequent ecdysone-meals, their conversion of normal bloodmeal into eggs was less effective compared to that of normal females. Their total oxygen consumption was also comparable to that of normal females during the period of post-bloodmeal digestion and oocyte maturation. Anatomical and histological comparisons revealed significant differences between the adjusted mean size indices (in terms of unfed weight) of normal and ecdysteroid treated females. Thus supermoulted females weigh less than would be expected for their size. These supermoulted ticks also had enlarged salivary glands and the salivary glands of twice supermoulted females were proportionally larger than those of normal or once supermoulted females. Cuticular patterns of normal and supermoulted adult females also showed differences; the number of mammillae per optic field became fewer as they grew larger and became more prominent with each subsequent moult. Supermoulted females also showed accumulation of salt crystals around their mouth parts which may imply that faulty water balance mechanisms contribute to their greater mortality in comparison to normal females.

From the foregoing observations it was concluded that:

1. For *in vitro* mass rearing, rabbits and chickens are better laboratory hosts than are rats and guinea pigs. The female ticks fed on rabbits and chickens can be expected to have higher levels of egg production. Nymphal ticks fed on rabbit and chicken hosts also show low levels of mortality coupled with rapid moulting and adult development.
2. Defibrinated porcine blood is an excellent substrate for the promotion of good *in vitro* feeding and high levels of egg production. Nymphs fed *in vitro* on this substrate had good developmental rates with early adult maturation. These positive effects of defibrinated pig blood make the *in vitro* breeding technique the method of choice for colony rearing and maintenance.
3. Adult male *O. p. porcinus* show significantly higher mortality responses to topically applied beta-ecdysone (at any particular dose level) than do adult females. Both sexes show low supermoulting responses to topical ecdysteroids with males responding at a slightly higher rate.

4. Topical application of ecdysteroids to nymphs will produce high mortality responses with the sensitivity of nymphs increasing significantly from day 1 to day 4 post-feeding. Since mortality was always less than 80%, other ecdysteroids should be tested at a range of practical dose levels. As a potentially successful growth regulator, any ecdysteroid must cause more than 90% mortality before it could be tested for potential in the field.
5. The moulting acceleration which was observed among the surviving nymphs is undesirable in terms of tick control.
6. At lower doses (1 to 4μg/ml), ingestion of ecdysteroids will cause supermoulting and low mortality among beta-ecdysone and ponasterone A fed ticks. Higher doses (5 to 10 μg/ml) cause mortality of up to 90% among both males and females. Ecdysone-fed ticks may supermoult as many as 3 times. *Ornithodoros p. porcinus* adults do not lose their supermoulting potential with increasing age and they can also respond to many other ecdysteroids.
7. Ingestion of beta-ecdysone or ponasterone A causes moderate to high mortality among nymphal instars. Because of the absence of a generally linear dose-response curve it is not now feasible to test either of these ecdysteroids as a systemic growth regulator for ticks. Ingestion of these ecdysteroids also causes nymphal moult acceleration among the survivors.
8. There are basic peaks of moulting hormone activity in the haemolymph of fed nymphs and supermoulting adults. The two peaks appear to be related to the moulting process. In normally-fed and mated females a peak of moulting hormone activity appears on day 7 and this seems to be related to egg maturation. Therefore ticks show titre changes in association with both moulting and reproductive cycles and may have endocrine patterns and mechanisms similar to those found in insects.
9. Supermoulted female *O. p. porcinus* take larger bloodmeals and lay more eggs than normal females. On a comparative basis, however, their conversion of bloodmeal into eggs is less efficient and they have shorter adult lives. The shortening of adult life seems to be related to physiological defects in their water balance mechanisms. Accordingly, the total egg production of supermoulted females might not be greater than that of the longer-lived and more efficient normal females.
10. There may be some potential for the development of an analogue to the natural tick ecdysone(s) which could be used for tick control by systemic administration through livestock hosts. Any such analogue would have to persist at relatively high levels in the host circulation and should not contribute to the supermoulting of adults or moult acceleration of nymphs.

Production of ecdysteroids by various tissues of adult *R. appendiculatus in vitro*.

B-J. Ellis, F. D. Obenchain, N. Ole Sitayo and R. Ojowa

Preliminary investigations at ICIPE show that tick moulting and reproductive development (oogenesis) are co-ordinated by the timing and magnitude of changing haemolymph titres of ecdysteroid hormones (Mango et al, 1977 and 1978 ICIPE Annual Reports). Similar, if not identical insect ecdysteroids (alpha- and beta-ecdysone) are involved in the control of these same processes in insects such as mosquitoes and tsetse flies. Because insects are naturally divided into three body regions (head, thorax and abdomen) it has been possible to perform ligation experiments which show that alpha-ecdysone is produced in the insect thorax. Ablation and transplantation experiments further indicate that prothoracic glands are the source of this hormone and these isolated glands will produce alpha-ecdysone *in vitro* (as determined by bioassay and chemical identification) under the appropriate culture conditions. Since ticks have a single body region which expands enormously during feeding it has not been feasible to study the source of tick ecdysteroids by ligation or ablation/transplantion experiments. Through the combined facilities of ICIPE and The International Laboratory for Research on Animal Diseases, however, it has been possible to bypass some of the experimental approaches used in the earlier insect work and to perform a preliminary investigation on the capacities of various adult tick tissues to synthesize and release ecdysteroids *in vitro*.

During a 6 week period in July-August 1979, rabbit-fed adult *Rhipicephalus appendiculatus* ticks from the ICIPE tick colonies (Chiromo and Muguga) were dissected at ILRAD and various tissue combinations were cultured in 100 μl of modified L-15 medium (with 1%, 5% or 20% added fetal calf serum) at 27 or 37°C. for 7 days. Ecdysteroid production was quantified by radioimmunoassay of 20μl aliquots of the incubation medium according to the procedures of Chang and O'Connor (1979) in the ICIPE Bioassay Research Unit (and counted in the ILRAD Biochemistry laboratory).

Detectable levels of ecdysteroid were only obtained for pooled cultures of tick fat body and attached tracheal trunks, salivary glands or central nervous systems with 20% added FCS. At 27°C the ecdysteroid production was quantified at 25 pg/μl of beta-ecdysone (ecdysterone) equivalent for fat body and 12 pg/μl for salivary gland cultures. At 37°C fat body produced 42 pg/μl and central nervous system produced 11 pg/μl. Combined cultures of tick heart and pericardial tissues (together with epidermal tissues, muscles and pieces

of Malpighian tubules) produced relatively stable levels of ecdysteroids at 27°C varying between 12 and 20 pg/µl of culture media at the three concentrations of FCS. At 37°C, however, ecdysterone equivalent production increased from levels below the detectable threshold at 1% FCS to 15 pg/µl at 5% FCS and to 35 pg/µl at 20° FCS. Ecdysteroid production was higher in ovarian cultures. At 27°C amounts increased from 35 to 40, and finally to 50 pg/µl at 1%, 5% and 20% FCS, respectively. At 37°C the increase in production was even more dramatic with increasing concentrations of FCS (12, 25 and 90 pg/µl, respectively). The highest production appeared to occur in cultures of midgut epithelium. It was later determined, however that much of the apparent production was due to a quenching effect of haemoglobin and its derivatives in the sample aliquots. This quenching appeared to reduce the counts by 25 to 40%. After an initial quench correction, ecdysterone production by midgut cultures at 27°C still appeared to vary from 16 to 215 pg/µl, with highest production at 5 and 20% FCS. At 37°C production was lowest at 5% FCS (21 pg/µl), increasing to 46 pg/µl at 1% FCS, and highest at 190 pg/µl at 20% added FCS. It seems likely that the actual production of ecdysteroids by midgut cultures is actually lower than these predicted amounts. No detectable levels of ecdysteroids were found in 20µl aliquots of the culture media for any of the incubating male tissues.

It is too early to know the significance of these observations. Since the developing oocytes of *Amblyomma hebraeum* ticks sequester beta-ecdysone (Diehl, 1979) it is possible that the activity recovered from ovarian cultures was due to the release of stored ecdysteroids from lysing cells. This possibility cannot be confirmed or rejected until the completion of fine structural studies. On the other hand, the ecdysteroid activity recovered from midgut cultures could be due to the activity of the layer of fat body which covers the midgut epithelium instead of the activity of midgut tissues themselves. When the radio-immunoassays of tissues cultured in other media are completed it should be possible to determine whether the addition of midgut contents (rabbit blood) influenced the higher production of ecdysteroids by providing necessary substrates (cholesterol) which were not present in appropriate concentrations in L-15 medium fortified with 1% FCS.

The findings of these experiments should have relevance in several fields. A further understanding of endocrine mechanisms regulating tick moulting and reproduction may contribute to ICIPE's goal of developing alternative methods for tick control. Recently, scientists at the Veterinary Research Division of the Kenya Agricultural Research Institute (Muguga) have shown that production of the kinete stage of *Theileria parva* in the midgut epithelium of nymphal *R. appendiculatus* ticks is related to the timing of moulting events (Young and Leitch, J. Parasit. in press). Since tick moulting is regulated by ecdysteroids it is possible that the *Theileria* are responding directly to critical changes in the titres of the tick hormones. Knowledge of the tissue sources of these tick hormones may contribute to the development of *in vitro* culture techniques for the various tick stages of *T. parva*.

Precocene induced delay in oviposition and effects of exogenous beta-ecdysone and juvenile hormone (JH-III) in *O.p. porcinus*.

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

Leahy and Booth (personal communication) showed that topical applications of precocene-2 in dimethylsulfoxide (DMSO) significantly depressed the fertility of female argasid ticks. They were unable to reverse these effects with large doses of JH-III but, more recently, Pound and Oliver (1979) were able to reverse such effects with small doses (1 to 10 µg in DMSO) in precocene-2 treated females of *Ornithodoros parkeri*. The following experiments were initiated to determine if exogenous beta-ecdysone and/or JH-III would reverse precocene induced sterility in *O. p. porcinus*. Females were fed *in vitro* on defibrinated pig blood and on blood containing 0.4 µg/ml added beta-ecdysone (an amount too low to induce supermoulting). Groups of 20 females from each feeding regimen were subjected to one of the following treatments immediately post-feeding:—

(1) groups were set aside as untreated controls.
(2) groups were dipped for 5 secs. in acetone, then topically treated with 2 µl of DMSO.
(3) groups were dipped for 5 secs in acetone containing 0.1 mg/ml of JH-III.
(4) groups were treated with JH-III as in (3) and then topically treated with 1 mg precocene-2 in 2µl of DMSO.
(5) groups were topically treated with 1 mg precocene-2 in 2µl of DMSO.

Thirty minutes after the above treatments, all females were put with recently fed males for mating. Four days after treatment the females were placed in individual vials with a male and observed daily for oviposition or death. Observed mortality reached 20% among precocene treated females, as compared to 0 to 10% mortality in the other groups.

The effects of exogenous ecdysone on rates of oviposition were not significant, with no apparent differences in the slopes of the plots (accumulative % oviposition on a probability scale versus days post-feeding) or in the time taken to reach the 50% level of accumulative oviposition. In Figure 4 the normally and ecdysone fed ticks are grouped for each of the other treatments. The plots for untreated and solvent control groups are essentially the same as that for the JH-III treated ticks, with the 50% accumulative level of oviposition occurring between days 17 and 19 post-feeding. Oviposition

began by days 16 to 20 post-feeding among precocene treated groups; but the plot for ticks treated with both JH-III and precocene has a more elevated slope than the plot for ticks treated only with precocene. The 50% accumulative level of oviposition was reached on day 30.5 after JH III and precocene treatment but the same degree of oviposition did not occur until day 36.5 after precocene only. Under these conditions exogenous JH-III seems to have partially reversed the effects of precocene.

As in the experiments on moulting delay (1978 ICIPE Annual Report) ovipositional delay appears to be related to a reversible inhibition of metabolism by a mechanism of general toxicity. During the period of delay, precocene treated ticks remained the same size and excreted no guanine, although the ovipositing non-precocene treated ticks were doing so. Those ticks which died soon after precocene treatment seemed to be suffering from a general paralysis and they died with their legs in an extended position. It would appear that the surviving precocene treated ticks were able to return to a "normal" pattern of reproductive development after the precocene had been metabolically neutralized. During that process, trace amounts of residual exogenous JH-III may have stimulated egg production.

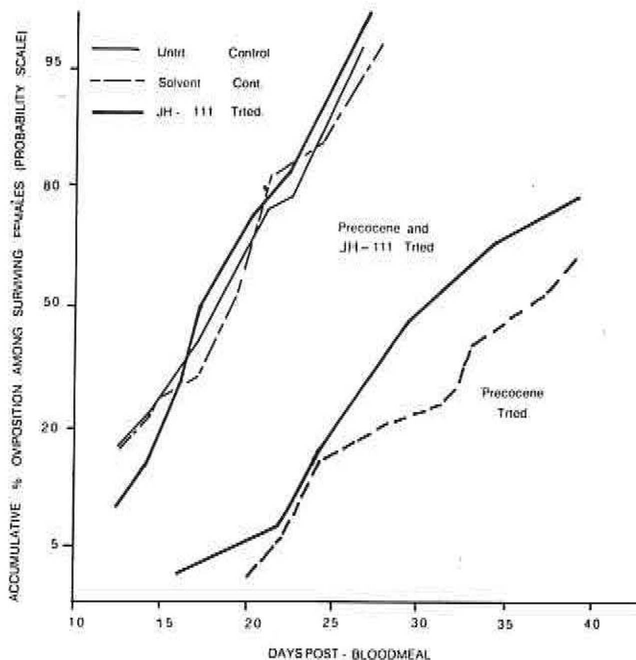


Figure 4. The effects of precocene–2 on ovipositional delay in *O. p. porcinus* and antagonistic action of JH–III.

Responses of Cattle to an extract from the Larvae of the Tick *Rhipicephalus appendiculatus*

G. M. Binta

Introduction

Resistance to larvae of the tick *Boophilus microplus* is a long established phenomenon in cattle. On a highly resistant animal, less than 1% of the original larval population successfully complete their life cycle. Within 24 hours of attachment, there is rejection of larval ticks. Thereafter, repeated attachment and detachment ensue. The rejection in a bovine host is attributed to an immediate cutaneous hypersensitivity reaction. Isolation of a DFP—sensitive esterase as allergen 1., capable of inducing an immediate hypersensitivity reaction in cattle exposed to *Boophilus microplus* has been reported. (Willadsen and Williams, 1976). Recently, another protein i.e. allergen 2. has been extracted from *B. microplus* larvae, (Willadsen, et al 1978). The aim of the present study was to partially purify an allergen from the larval ticks of *Rhipicephalus appendiculatus* and then use the allergen to elicit an immediate cutaneous hypersensitivity type reaction. Thus the skin reaction could then be correlated to the status of resistance to *R. appendiculatus in cattle*.

Materials and methods: ticks

Larval ticks of a *Rhipicephalus appendiculatus* strain were obtained from the Veterinary Research Department Laboratories at Muguga.

Purification of allergen 2

A typical purification has been described by Willadsen, *et al* (1978). Six and a half grams of larval material was used. The material was concentrated using Polyethylene glycol 6000 sprinkled over the material packed in dialysis tubings.

Protein determination

The method of Warburg and Christian (1941) was used.

Animals

Two steers of exotic breed with a history of previous intensive exposure to ticks and two calves with no previous exposure to ticks were used. The two steers were K326 and L049 while the calves were numbered as N238 and M970.

Immediate hypersensitivity tests

These were performed as recommended by Willadsen and Williams (1976), using a single concentration of the extract. The protein concentration of the material was 1mg/ml.

Results

The results are as depicted in Tables 5 and 6.

Table 5. Purification of allergen 2

	Fraction	Total Protein
(a)	Crude Extract	22.8 mg/ml.
(b)	50% (NH_4SO_4) after dialysis	15.5 mg/ml.
(c)	DEAE eluate after concentration	50 mg/ml.
(d)	Allergen 2, after CM Cellulose Chromatography	1 mg/ml.

Discussion

The two steers K326 and L049 with a previous exposure to instars of *Rhipicephalus appendiculatus* elicited an immediate cutaneous hypersensitivity reaction, within 20 minutes. This response to the extract was characterized by a bullae formation at the site as a result of oedema. This was painful to the touch. The bullae in L049 receeded within 6 hours. Absence of a reaction in the calves and appearance of a bullae after some time (16 hours) tends to indicate a difference in the resistance levels.

Table 6. Changes in skin thickness expressed as millimetres in response to the larval extract from *Rhipicephalus appendiculatus*

	Animal No.	N238	M970		K326a		L049a	
Time in minutes	Test	Saline b	Test	Saline b	Test	Saline b	Test	Saline b
0 min.	7	7	7	7	7;7	5;6	7;77	8
20 min.	7	7	7	7	12;10	5;6	10;11;10	8
1 hr.	· 7	7	7	7	12;10	5;6	11;11;11	8
5 hrs.	NM	NM	NM	NM	14;12	5;6	12;11;11	8
16 hours	10	7	10	7	12;12	5;6	8 8 8	8
24 hours	10	7	10	7	12;12	5;6	7.5;7.5;7.5	8
72 hours	10	7	10	7	12;12	5;6	7;7;7	8
96 hours	10	7	10	7	10;10	5;6	7;7;7	8
1 week	7	7	7	7	7;7	5;6	7;7;7	8

N.B. a. K326 had 2 test sites and L049, 3.
b. Saline used as control inoculum.

From the history of the calves, it would be valid to suggest that the calves were not as resistant to ticks as L049 and K326. Recession of the bullae in L049 could indicate that this steer was more resistant than K326. Standardisation of this technique for the extract and skin testing of several cattle are required before the method can be adopted to pin-point resistant cattle.

Visiting Director of Research
Professor J. Mouchet (1975)

Programme Leader
Dr. R. Subra (1978)

Research Staff
Mr. P. Amutalla (1979) Technical Assistant
Mr. N. M. Komeri (1979) Junior Technician
Dr. A. W. R. McCrae (1977) Senior Research Scientist
Dr. J. B. Kaddu (1979) Postdoctoral Research Fellow
Mr. E. Mkuzi (1976) Technical Assistant
Dr. F. W. Mosha (1976) Postdoctoral Research Fellow
Mr. S. Muti (1978) Junior Technician
Mr. C. M. Mutero, Research Trainee, University of Nairobi
Dr. M. J. Mutinga (1979) Senior Research Scientist
Mr. P. M. Mwamisi (1978) Technical Assistant/Driver
Mr. J. Mwandandu (1971) Tedhnical Assistant/Driver
Mrs. N. M. Ouna (1979) Principal Technician

COMPETITION STUDIES BETWEEN *CULEX PIPIENS QUINQUEFASCIATUS (=FATIGANS)* AND *CULEX CINEREUS*

R. Subra

Culex pipiens quinquefasciatus, a major filariasis vector, develops in man-made breeding-places: cesspools and latrines which are flooded by the water table. In some areas along the Kenya Coast where it has been present for many years it is found breeding alone, while in areas further inland where it has been reported fairly recently (less than 10 years ago) it breeds with another mosquito species, *Culex cinereus*. In that case *C. p. quinquefasciatus* is not able to maintain itself very long and it disappears, leaving *C. cinereus* in those breeding-places. Should the elimination of *C. p. quinquefasciatus* be due to the presence of *C. cinereus*, the introduction of this mosquito to *C. p. quinquefasciatus* breeding-sites could be a way of controlling this filariasis vector. In order to check this hypothesis experiments and observations have been made both in the laboratory and in the field.

In the laboratory both species were bred together and alone (for control) at different densities and proportions. The commencing densities of first instar larvae per cm^2 of water surface were: 1.25, 2.50, 5.0, 10.0, 20.0 and 40.0. The proportions of *C. p. quinquefasciatus* and *C. cinereus* used at each of these densities were, 100:0, 75:25, 50:50, 25:75, 0:100 respectively. The aim of these experiments was to assess for each species 1) the yield of pupae, 2) the time needed for all larvae to pupate. The results may be summarized as follows: The yield of *C. cinereus* pupae is very high and almost constant. It seems to be independent of the densities and proportions of the two species. In contrast, the yield of *C. p. quinquefasciatus* pupae depends on the densities and proportions of the two species. The higher the densities and the proportions of *C. cinereus* the lower the *C. p. quinquefasciatus* yield. As far as pupation is concerned it has been observed that when the two species are bred separately the first appearance of pupae and also the pupation peak occur at about the same time for each species. Nevertheless, the time needed for all larvae to pupate is longer for *C. p. quinquefasciatus* than for *C. cinereus*. When the two species are reared together the pupation peak occurs earlier in *C. cinereus* than in *C.* p. *quinquefasciatus*. As densities are increased the higher the proportion of *C. cinereus* the more delayed the *C. p. quinquefasciatus* pupation peak. At highest densities the pupation of *C. p. quinquefasciatus* starts only when almost all *C. cinereus* larvae have already pupated. These results are indicated in Figure 1 for densities of 20 larvae/cm^2. From these experiments it may be concluded that *C. cinereus* affects both the yield and the development rate of *C. p. quinquefasciatus*.

Field observations were made in a breeding-place (latrine) where the two species occurred together. Results reported here concern adult mosquitoes caught when leaving the latrine, after the 2 species were well established in the breeding-place. At a preliminary stage, sampling was done on cycles of 24 hours in order to know at which time the different categories of adults were leaving the breeding-place, and whether from the data so collected it was possible to sample only at certain periods of the day. The different adult categories considered were as follows: *C. p. quinquefasciatus:* emerging males and females, unfed parous females, i.e. females which had laid eggs, and gravid females, i.e. females which were leaving the breeding-place without having laid eggs. *C. cinereus:* only emerging males were considered, the distinction between newly emerged and unfed parous females being too time-consuming. Fig. 2 shows that most of the newly emerged adults of *C. p. quinquefasciatus* (males and females) depart from the breeding-place late in the afternoon. Unfed parous females show two peaks, one at sunset and another, the most important, at sunrise. Gravid females also show two peaks at the same periods, but of these, the sunset peak is the greater. From these observations it was

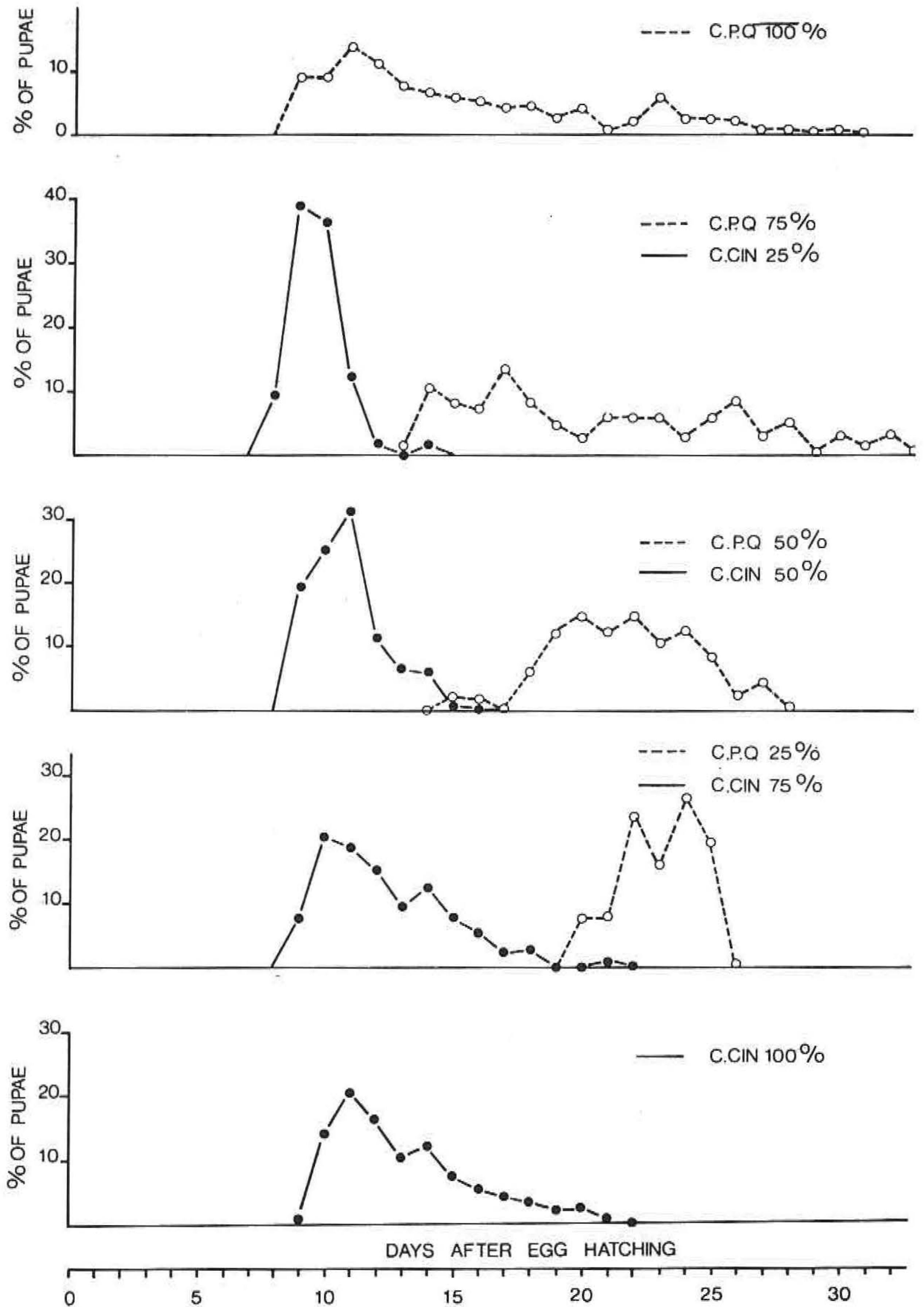


Figure 1. Pupation rate of *C. p. quinquefasciatus* and *C. cinereus* at an orginal density of 20 1st instar larvae/cm².

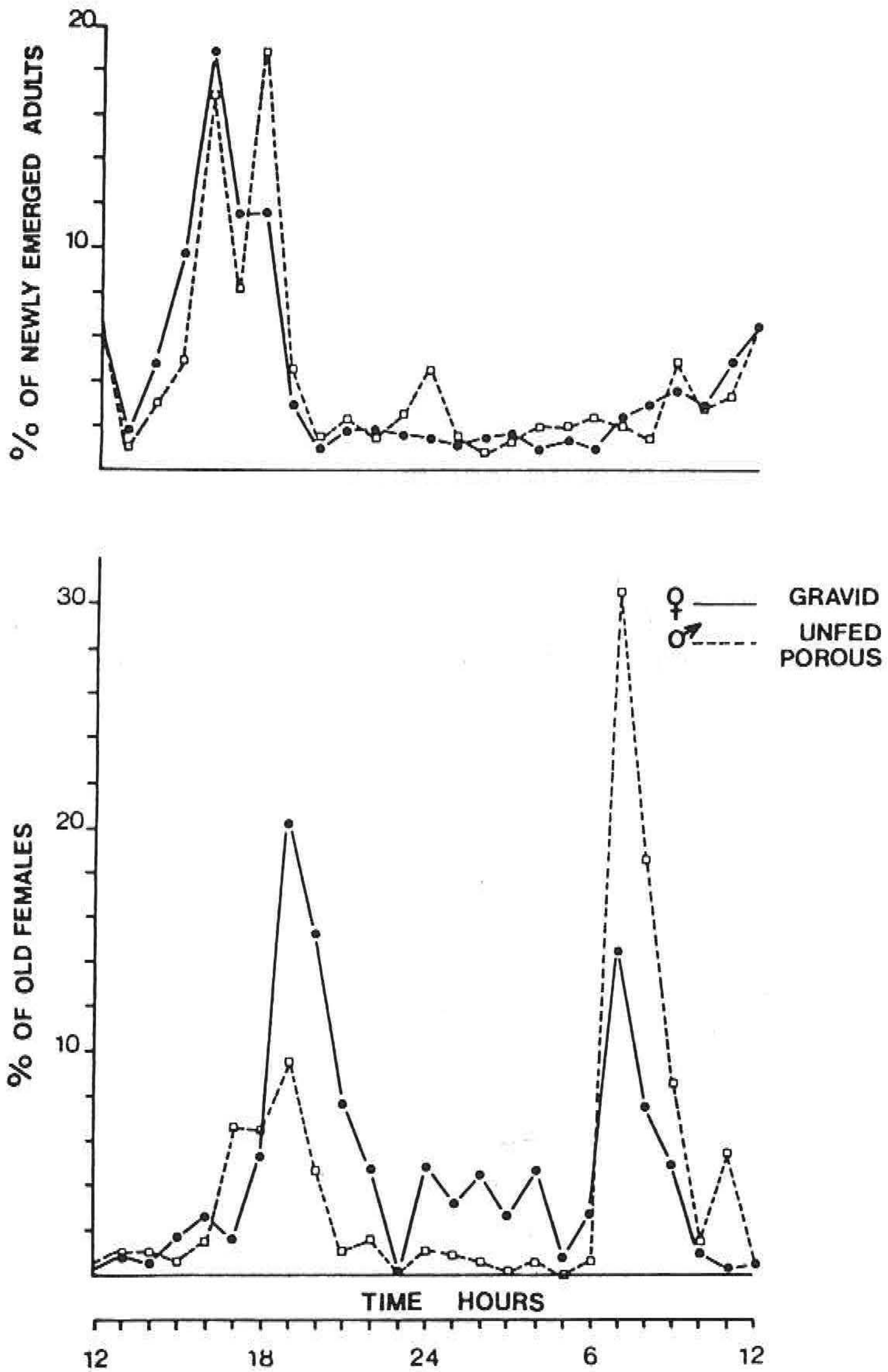


Figure 2. Daily exodus from a pit latrine of *C. p. quinquefasciatus* adults.

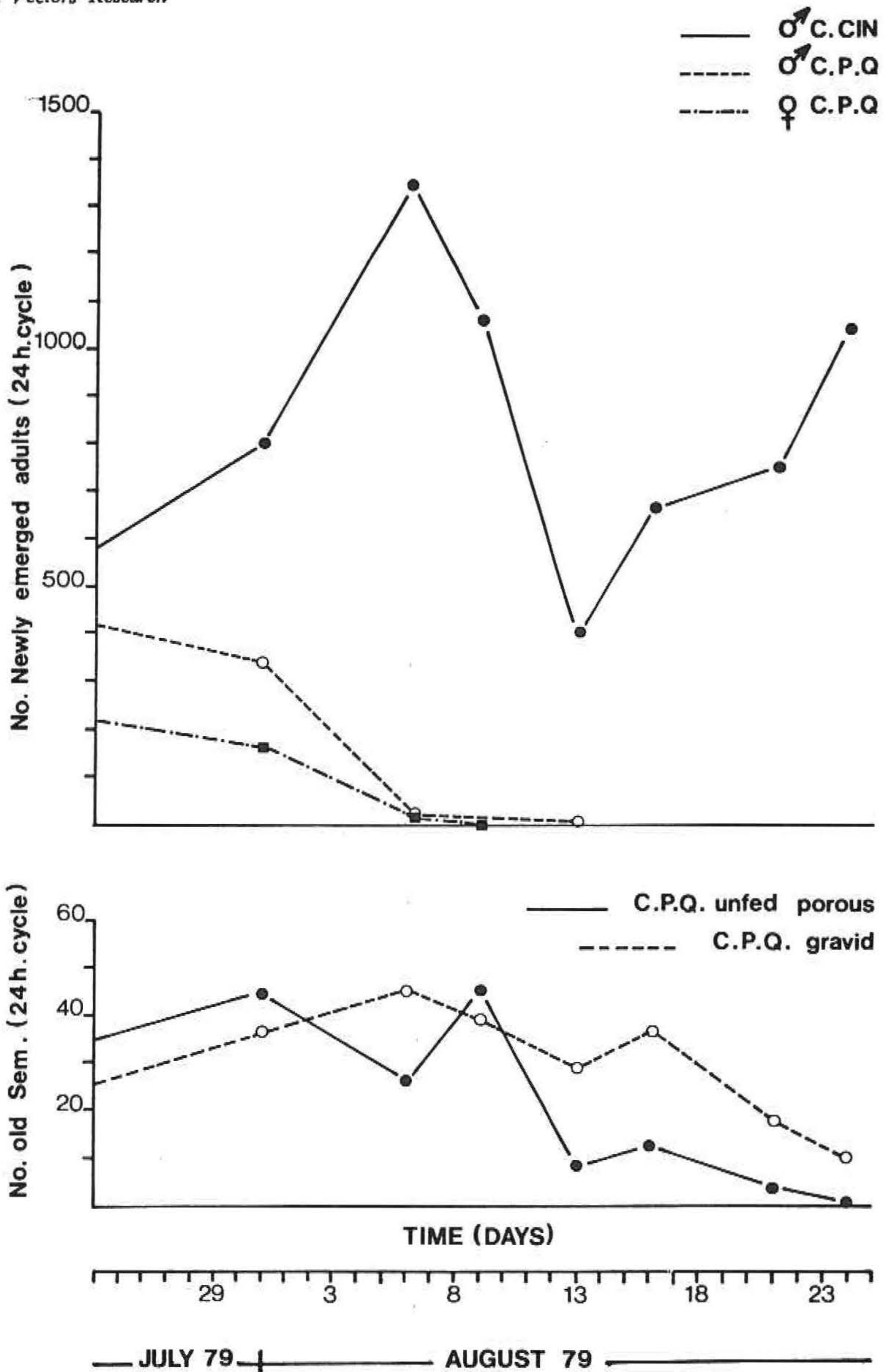


Figure 3. Densities of *C. p. quinquefasciatus* and *C. cinereus* adults departing from a pit latrine in which both species initially occurred together.

concluded that sampling had to be conducted on full 24-hour cycles. Trapping entering females showed that only gravids of *C. p. quinquefasciatus* were attracted to the breeding-place.

Adult sampling was initiated a few weeks after the two species had become established in the breeding-place. Throughout the time that the observations were made *C. cinereus* densities remained high while *C. p. quinquefasciatus* declined, disappearing after 2 weeks (Fig. 3). Nevertheless during the next two weeks a certain number of unfed parous *C. p. quinquefasciatus* females were collected leaving the breeding-place after laying eggs. Thus even when the site no longer produced adult *C. p. quinquefasciatus*, eggs of this species were still being introduced into it. Field observations therefore confirm laboratory ones: that in the presence of *C. cinereus*, *C. p. quinquefasciatus* is unable to maintain itself in the same breeding-place.

Additional observations were made on the relative proportions of unfed parous females (i.e. those which had laid eggs in the breeding-place) and on gravid females (i.e. those which were leaving without laying eggs). While the breeding-place was still producing *C. p. quinquefasciatus* adults, the proportion of unfed females was higher. After the adult production stopped, gravids became dominant and the number of unfed ones became insignificant. Even if the challenge of *C. cinereus* larvae was the major reason for the disappearance of *C. p. quinquefasciatus* from the breeding-place, a secondary reason seems to have been a declining attractiveness to ovipositing *C.p. quinquefasciatus* for a site occupied by *C. cinereus*.

Further studies will be initiated to find out the reasons why *C. cinereus* is able to settle only in specific breeding-places and not in others.

Anopheline Ecology

A. W. R. McCrae

Research continues to be concentrated chiefly on the freshwater breeding species of the *Anopheles gambiae* complex, vectors of approximately ⅓ of the world's current malaria, with the aim of identifying naturalistic methods of control.

Oviposition Studies

Direct observation of behaviour

Wild-caught *A. gambiae* in cages 60 cm in each dimension were observed in red light. For closest detail, mosquitoes in a smaller observation chamber were viewed laterally by stereomicroscope. Principal findings were:

(a) Preliminary behaviour takes the form of distinctive, rapid, backwards-looping flights over water surfaces.
(b) The female may then land and commence oviposition immediately or fly off, often to rest, before returning to oviposit with or without further preliminaries.
(c) After this, oviposition sometimes proceeds without tarsal water contact, e.g. from lip of petri dish or wall of observation chamber.
(d) Most eggs are laid at intervals of 5 to 8 seconds.
(e) The full course of oviposition is usually interspersed with up to 8 interim bouts of flying or of restless grooming and walking.
(f) Eggs are rarely scattered from flight, as confirmed by experiments.
(g) Allowing for (e), a normal full complement of 100 to 265 eggs is laid in some 15 to 35 minutes.
(h) Throughout oviposition, no part of the mosquito but its tarsi touches the substrate.

Circadian activity pattern

On the day of capture, blood-fed mosquitoes were released into 60 cm cube cages well before sunset and left undisturbed. From next sunset, 4 petri dishes of water were presented and changed hourly.

Results from 10 nights are compared in Figure 4 with those from the only previous substantial study by Haddow and Ssenkubuge (1962) *Ann. trop. Med. Parasitol.* **56**: 352–5, who used a long established insectary strain of *A. gambiae sensu stricto* to which they offered blood for a short period. In the present study, *A. gambiae* (almost all if not entirely s. str.) would not have been adapted to insectary conditions, and blood feeding would have followed a natural pattern.

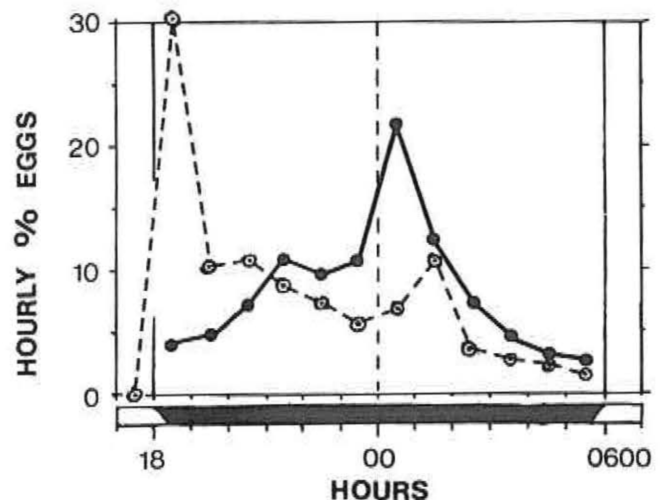


Figure 4. Circadian pattern of oviposition activity by freshwater *A. gambiae*, showing the percentage of total eggs for each of the two series laid each hour.

Thick line: results of the present study using wild-caught mosquitoes (10 nights; 24, 176 eggs).

Dashed line: results of Haddow and Ssenkubuge (1962) using an insectrary-adapted strain of mosquitces.

To indicate whether the pattern of oviposition may have an endogenous basis and not be merely a function of time elapsing after blood-feeding, gravid females were held for an additional full day before allowing them to oviposit. Results from 4 nights were skewed by heavy mortality, yet the peak fell not in the 1st but in the 2nd to 4th hours of the night.

These results underscore the need to use wild strains in behavioural studies.

Oviposition site selection experiments

Following up last year's preliminary studies, dark-coloured swamp water (in which *A. gambiae* larvae are never found in nature) was tested in a 4×4 array in a large cage set up in the field against 2 types of actual breeding site water and distilled water all in white plastic dishes. Very strong oviposition preference was indicated for the dark swamp water. To test this result on free-ranging mosquitoes, the same swamp or puddle water was put into 20 freshly-dug pits, in which *A. gambiae* then oviposited indiscriminately. Multiple factors were evidently at play, calling for experimental procedures allowing factors to be tested singly.

All subsequent experiments have therefore been indoors in 60 cm cube cages floored with either black, white or grey (mid-tone) card. Oviposition targets were 9 cm glass petri dishes placed on 9 cm cards of the above tones. After full night experiments the mosquitoes were examined individually and dissected for retained eggs as necessary. Basic test procedures were:
(a) Alternative choice with 2×2 layout, or
(b) A special 3 (treated) ×1 (untreated) layout as test for repellency, where the single untreated dish was in the least preferred position, receiving few if any eggs when all dishes were untreated.

In the following experiments, freshwater *A. gambiae* were used in dim indirect lighting unless otherwise stated. Eggs were laid in groups of unknown variance, and interpretation therefore rests on consistency between replicate sets.

The following experiments are listed according to the major stimuli/responses involved.

(a) Visual

(i) Black vs white targets with all-white surrounds. *A. gambiae* laid 83.25% and *A. funestus* 99.8% of eggs on black targets.
(ii) Tone density of targets and contrast with surrounds. Black is always preferred (Figure 5).
(iii) Positional preference: effects of direction of illumination. Targets closest to the illuminated side of a cage are preferred.
(iv) Tone density vs positional preferences at 3 light intensities. Positional effects continue to be expressed at lowest illumination while tone density effects wane.
(v) Is oviposition inhibited by total darkness? Evidently not at all.

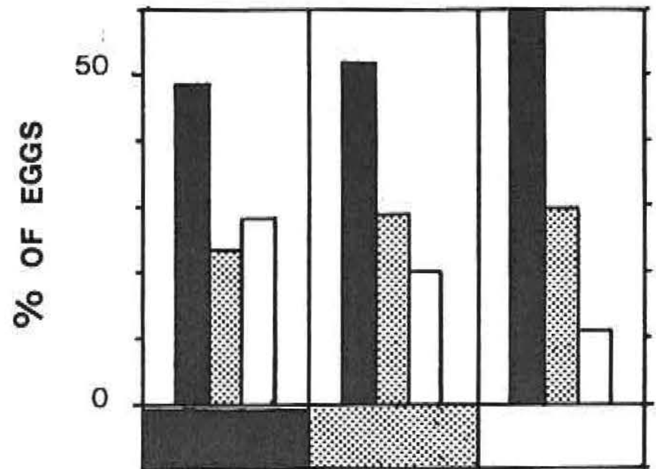


Figure 5. The mean average percentages of eggs laid by freshwater *A. gambiae* in 3 petri dishes each backed with a disc of black, grey or white against a cage floor of one of the same tone densities, as indicated above. Results show the relative intrinsic and contrast effects on oviposition of target and background tone, based on competition between dishes. Total eggs: 35,583.

(vi) Physical obstructions. (a) Inverted test tubes within white dishes. *A. gambiae* laid 63.4% and *A. funestus* (though inhibited) 85.3% in obstructed dishes. (b) with 6 further tubes outside the dish, more eggs went to unobstructed dishes.

(b) Tactile

(i) Free water surface vs damp surface (white absorbent paper) in all-white cages. *A. gambiae* laid a mean of 70.8% of eggs on free water. Two repeats in total darkness yielded a mean of 60.05% for free water, indicating relative influence of visual and tactile stimuli. In dim light, *Anopheles merus* laid only 43.4% of eggs on free water, but even using 25% sea water, this species showed great reluctance to oviposit on white targets.
(ii) Is initial tarsal water contact necessary for oviposition? In dishes screened with fine black netting 5mm above the surface, no eggs were laid.

(c) Chemical treatment

(i) Tokens of water maturity: little or no discrimination evident with (a) Nitrite (representing rainwater) 0.01% N_aNO_2 vs dechlorinated tap water. (b) Ammonia (representing "mature" to polluted waters): 0.02% NH_2 vs same.
(ii) Sea water vs tap water. Though eggs of freshwater *gambiae* are rapidly killed in sea water, 26.5% of eggs were laid in it. Some individual females seemed indifferent.
(iii) Phenol. 1.0% phenol inhibited all oviposition; 0.1% gave results suggesting damage to chemoreceptors, perhaps helping explain sea water results.

(d) Natural waters

Swamp water vs dam (breeding-site) water, on all-black background. Results inconsistent, indicating only slightly

greater attractancy (or less repellency) of the latter, as with swamp vs tap and tap vs dam water.

(e) Surface activity

If pre-obstetric dances involve testing only water surface, might surface tension be of prime importance? 3 × 1 repellency test procedures were used here:
(i) Oiling: "HS malariol" a standard larvicide, at 3 ml per cm² of water surface showed some repellency.
(ii) Soya lecithin. Thick layers repelled.
(iii) Surfactants. (a) A 1.0% solution of the odourless sodium lauryl sulphate drowned some 70% of ovipositing mosquitoes. Repellency was not evident. Many eggs were laid on the dry (black) cage floor, presumably mediated by prolonged wetting effect on tarsi. (b) 1.0% teepol drowned more than 90% before any oviposited. The remainder apparently abstained. Results were as if the solution attracted. Teepol is now being used in initial trials for field sampling.

In sum, these studies indicate that visual responses override chemosensory ones provided water is not grossly contaminated. Some pitfalls of cage experiments have been clarified, but interpretation of these results in terms of free-ranging behaviour must remain very tentative. All ideas so derived need field confirmation.

Egg ecology

Stranding. This seems the major cause of egg loss in nature. In the laboratory at 70–80% RH on damp surfaces, stranded eggs remain unhatched until wetted up to 6 days after laying; after that, viability declines rapidly. On wet surfaces, eggs either hatch and the larvae suffer high mortality or the eggs may hatch up to 10 days from laying. Even so, there is a marked though lesser decline in viability after 6 days. Relevant field evidence is shown in Fig 6.

Field location of eggs. Reappraisal of past and current experience and of the many published reports indicate one single factor which virtually all breeding sites of freshwater *A. gambiae* share, viz:— the water margin is clear of dense vegetation. Other "typical" features such as recent formation and high turbidity seem consequences of predation. The site-seeking female would therefore be expected to be arrested by features of the edge, which at larger bodies of water may lead to localised egg concentrations. This seems an extremely important point and will be pursued in next year's field programme.

Aquatic population regulation

Ideal conditions for this study were encountered in January at a small permanent dam 15 km directly west of Mombasa. Sampling proceeded from 26 January for 60 consecutive mornings, supplemented by many other field and laboratory investigations. This continues by weekly sampling extended to include representative breeding sites from a hilltop to a valley drainage line. Adult mosquito populations have also been monitored throughout.

Sampling for each of the first 60 days was at 0800–0930 h from 6 separate points along the dam shore line by a fixed quadrat method covering a daily total of 300 cm² of water surface. Total yields of *A. gambiae* larval instars and pupae are shown in Table 1. To determine development durations and any daily patterns of moulting times, samples were taken at more frequent intervals. Until heavy rain fell at night these showed no consistent patterns, even when larvae had been graded to 6 development stages for each of the first 3 instars and to 4 for the 4th, based on head capsule changes. However, the clear patterns shown in Figure 6 are fully substantiated by these intra-instar growth stage grades. Durations of 1st to 3rd instars shown in Table 1 are derived from this; of the 4th instar and pupa, from other findings at the dam.

Table 1 shows a deficit of 1st and 2nd instar larvae, a consequence of aggregation. Peak numbers of 3rd instars fell to about half in the equally aggregated 4ths followed by a dramatic 47-fold drop to the pupae. Sampled ¾-way through pupal life, further considerable losses of pupae seem inevitable by the time of adult emergence at nightfall. Latent effects cannot be responsible for these great pupal losses since high proportions of 4th instars kept in dam water will pupate within hours, followed by virtually complete survival to adult emergence. For this and other reasons the following causes may be excluded: (i) algal blooms or other surface effects (ii) pathogens (iii) Toxic factors in the water (iv) growth or moulting inhibitors, or other effects of crowding (v) upper lethal temperatures (vi) larval cannibalism.

The one obvious remaining factor is predation, yet 24 h sampling and direct observation have provided no evidence. The 4th instar larvae and pupae have similar microdistributions at all times. Of 13 potential predator species coexisting in shallow water with anophelines, all showed strong preference for larvae when tested in the laboratory and in field supplementation trials. The literature provides no further guidance. Studies continue, but until an answer is found, no one can advise on rational vector management in this vitally important context.

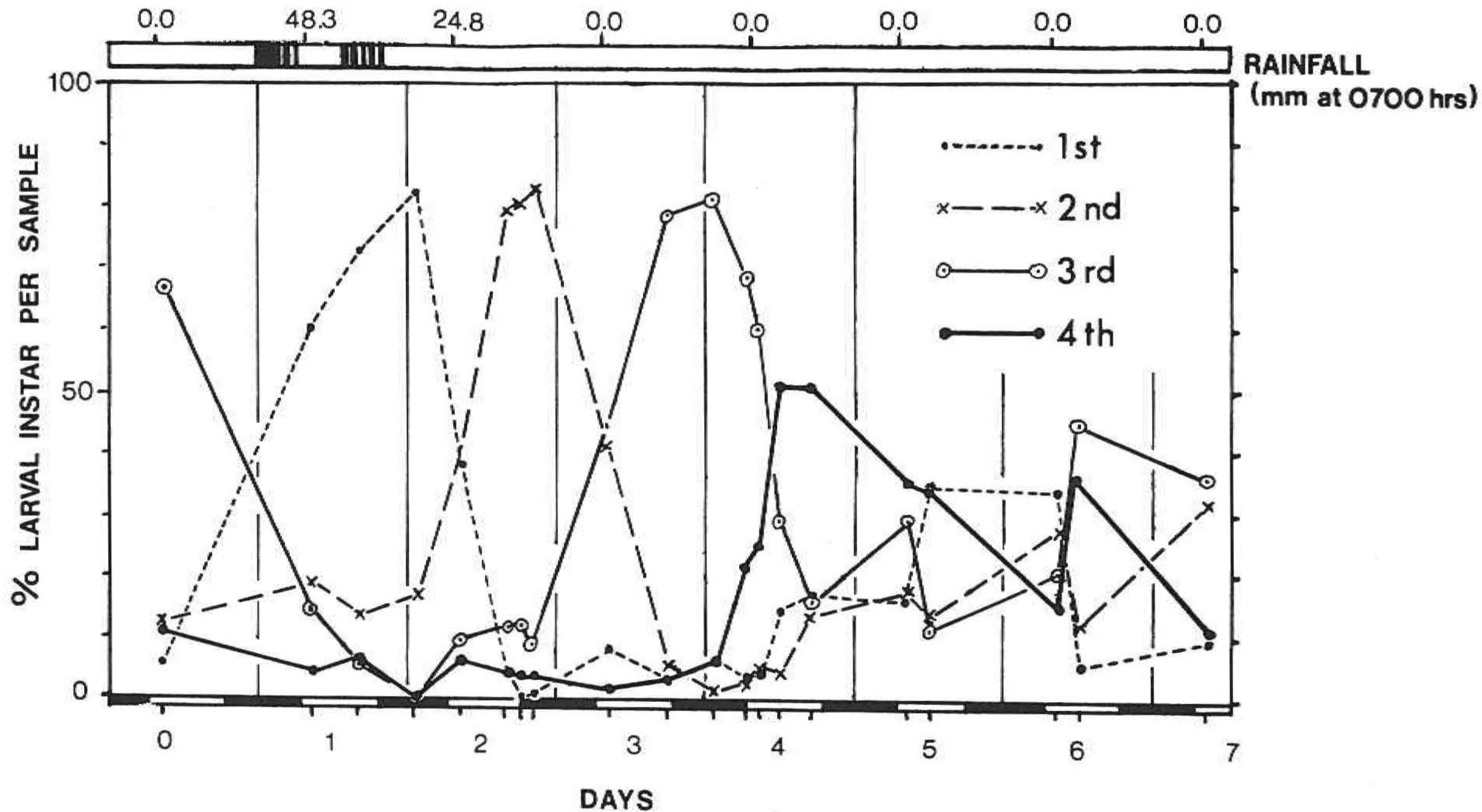


Figure 6. Percentages of each larval instar of *A. gambiae* from a sequence of 20 samples taken at irregular intervals over 8 days from a permanent dam near Mombasa. The duration and amount of rainfall are shown above, none had fallen int he previous 7 days. Note especially the short interval between rainfall and increase of 1st instars, for shorter than the 36–40h normal between oviposition and hatching at the prevailing temperatures. Note also the synchronous development of this cohort, followed by confusing incidence as unsynchronised cohorts enter the population.

Table 1. Numbers, proportions and adjusted proportions of aquatic stage *A. gambiae s.l.* from 60 consecutive days' sampling 0800–0930h from a permanent dam near Mombasa covering 300 cm² daily

	Larval instar				Pupae	Total
	1st	2nd	3rd	4th		
Total	1,070	2,979	5,275	2,441	36	11,801
Average total per day	17.83	49.65	87.92	40.68	0.60	196.68
Mean daily average % (A)	10.04	25.94	42.98	20.61	0.44	100.01
Estimated mean duration in hours (B)	? 26	24	26	? 28	27	131
A adjusted for *B*	9.98	27.92	42.68	19.00	0.42	100.00

ECOLOGY AND VECTORIAL EFFICIENCY OF *ANOPHELES GAMBIAE* SIBLING SPECIES

F. W. Mosha and C. M. Mutero

Introduction

Ecological studies on *An. gambiae* sibling species in relation to the transmission of malaria and of Bancroftian filariasis which were initiated in August, 1978, continued during the whole of 1979 in Jimbo and in one other village, Jego, located three kilometres inland from Jimbo on the South Kenya Coast. During this period, different methods for identification of *An. gambiae* sibling species were evaluated. The selection of Jego village with a predominant fresh water breeding *An. gambiae*, facilitated studies on comparative feeding and resting habits of *An. merus* and fresh water breeding *An. gambiae*.

Species composition

Table 2 shows average number of bites per person per night by different mosquito species. *Culex sitiens* was the next most abundant species after *An. gambiae sensu lato*, and included two other related species, namely *Cx. thalassius* and *Cx. tritae-niorhychus*. Other species which appeared sporadically in small numbers included *An. funestus*, *An. pharoensis*, *Culex cinereus*, *Cx. nebulosus*, *Aedes aegypti* and *Eretmapodites chrysogaster*.

An. gambiae complex

Identification

Attempts were made in separating members of the *An. gambiae* complex by using the following methods:—

(a) Salt tolerance test, as devised by Muirhead—Thomson
(b) Morphological characters on the palps and antennae
(c) Cytogenetic method involving the reading of polytene chromosomes prepared from developing ovaries.

Salt tolerance test involved the exposure of newly hatched larvae (from wild females) to 75% sea water for two hours. The larvae of adult mosquitoes that died within the two hours were considered as fresh water breeding *An. gambiae* while those that survived were considered as *An. merus*.

Between February and November 1979, 1171 *An. gambiae* out of 6168 total human bait collected mosquitoes (representing 19%) were identified by this method. Table 3 shows that 91.5% of outdoor collected *An. gambiae* was composed of *An. merus*, while for the indoor collection this species constituted 81.2% of the total *An. gambiae* tested.

The morphological characters were found not reliable enough for separation of *An. gambiae* sibling species. A total of 103 *An. merus* (separated by salinity test) from human bait collections carried out between February and May 1979 showed a higher mean palp index (0.86±0.04) and a higher mean number of coeloconic sensillae per antennae (23.3±4.79) than fresh water breeding *An. gambiae* which had mean values of 0.82±0.06 and 20.9±4.05 for palp index and coeloconic sensillae, respectively. When the mean values of palp index and coeloconic sensillae were plotted on a scatter diagram, a large overlap between the two characters was realised (Fig. 7) suggesting that these characters were of little taxonomic value. However, further studies are being carried out to find out if there are seasonal variations in these characters.

During this same period, the four-banded palp character which has been found useful in separating *An. melas* from fresh water breeding *An. gambiae* in West Africa was found in 41% (103/251) of *An. merus* and in 19.2% (10/52) of fresh water breeding *An. gambiae*. It therefore appeared not to be a conclusive character for separating *An. merus* from fresh water breeding *An. gambiae* in this area.

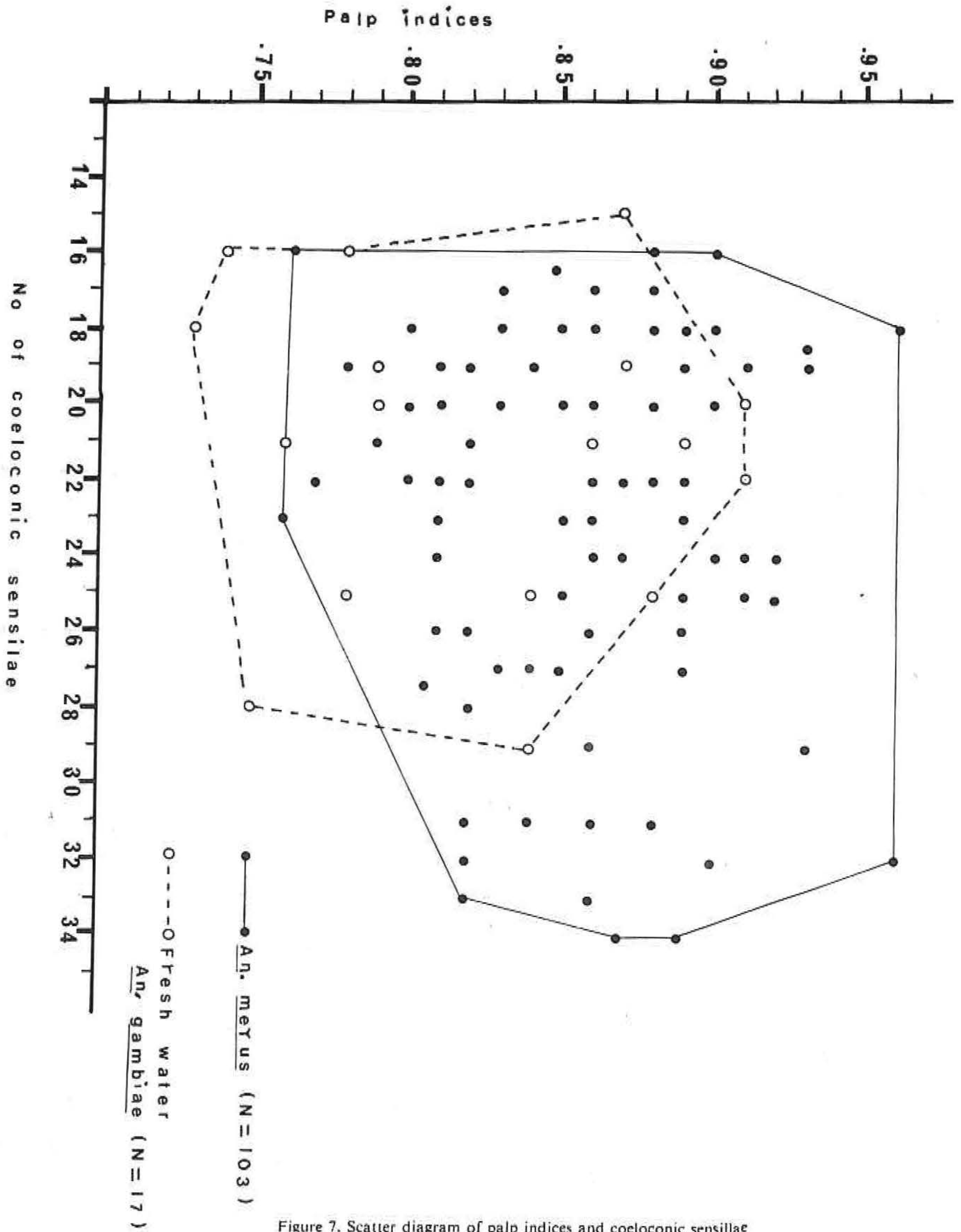


Figure 7. Scatter diagram of palp indices and coeloconic sensillae in *An. merus* and fresh water breeding *An. gambiae* collected from Jimbo village.

Table 2. Average number of mosquito bites per person per night in Jimbo between February and November, 1979

	Outdoor	Indoor
Anopheles gambiae	71.9	51.4
An. tenebrosus	0.5	0.1
Culex sitiens	31.3	9.7
Cx. quinquefasciatus	7.4	20.9
Aedes pembaensis	2.0	0.9
Ae. albocephalus	0.2	
Ae. sudanensis	0.1	0.04
Mansonia uniformis	5.1	
M. africanus	0.3	1.2
Others	1.3	1.1

Identification of *An. gambiae* sibling species by a cytogenetic method carried out later on by Dr. Coluzzi of the University of Rome gave proportions of *An. merus* and fresh water breeding *An. gambiae* for outdoor and indoor numbers almost similar to those obtained with salt tolerance tests (Table 3). Percentages of *An. merus*, *An. arabiensis* and *An. gambiae sensu stricto* were 98.2%, 1.2% and 0.6% respectively for outdoor collection, while for the indoor collection the respective percentages were 93.0%, 5.6% and 1.4%. No chromosomal polymorphism was observed in *An. merus*.

Breeding habits of *An. merus*

Preliminary laboratory investigations showed that *An merus* larvae develop well in 10%-20% sea water. Laboratory and field observations showed that *An. merus* was capable of undergoing full development in fresh water. Weekly measurements of salinity in a large pond in Jimbo after the rains had stopped showed an increase in salinity from 5.4% to 34% sea water between August and October 1979 mainly due to evaporation. There was a corresponding increase in the number of *An. gambiae s.l.* larvae and proportion of *An. merus* adults. Number of *An. gambiae s.l.* bites per person per night increased from 2.8 in August to 44.8 in October, 1979.

Feeding habits

In Jimbo, the peak activity period was observed in the first part of the night, while in Jego it was observed in the second part of the night, suggesting a difference in behaviour between *An. merus* which was predominant in Jimbo (over 90%) and fresh water breeding *An. gambiae* which was predominant in Jego (89%). More observations are being carried out to check whether there are any seasonal variations in this phenomenon. Results for *An. gambiae* complex from Jimbo suggest that *An. merus* feeds more outdoors than indoors.

Resting habits

Table 4 shows that there were more hand collected *An. gambiae s.l.* from Jego ($\epsilon\bar{x} = 6.3$) where fresh water breeding *An. gambiae* was predominant than from Jimbo ($\epsilon\bar{x} = 2.2$) where *An. merus* was predominant, suggesting stronger exophilic tendencies in *An. merus* than in fresh water breeding *An. gambiae*. High exophily in *An. merus* is also shown by comparatively low numbers of semigravid *An. gambiae s.l.* per house and absence of gravid *An. gambiae s.l.* in Jimbo.

Investigations for outdoor resting sites for different members of *An. gambiae* complex are currently under way in the study area. Drop nets and mechanical aspirators are being used to sample mosquitoes resting under fallen coconut leaves, roots of fallen coconut trees, heaps of coconut fruit fibres, grass and in mangroves. Fallen coconut leaves and heaps of coconut fruit fibres are proving to be the most favourable sites for *An. merus* outdoor resting.

Natural and Experimental Infections

Infection and infectivity rates of 0.3% ($\frac{4}{1171}$) and 0.2% ($\frac{2}{1171}$) respectively were observed in *An. merus* while in fresh water breeding *An. gambiae* the respective rates were 1.2% ($\frac{2}{173}$) and 0%. Dissections of *An. merus* 14 days after feeding on a human volunteer with an average of 62 microfilariae per 0.1 ml finger prick blood showed an infectivity rate of 47.2% ($\frac{34}{72}$).

Table 3. ***Anopheles gambiae*** **sibling species colleted by human bait method from Jimbo between February, and November, 1979**

Identification method	Species	Outdoor		Indoor	
		No.	%	No.	%
Salinity test	*An. merus*	704	91.5	467	81.2
	Others	65	8.5	108	18.8
	An. merus	164	98.2	66	93.0
Chromosomes	*An. arabiensis*	2	1.2	4	5.6
	An. gambiae	1	0.6	1	1.4

LEISHMANIASIS RESEARCH

M. J. Mutinga

Visceral leishmaniasis (Kala-azar) and cutaneous leishmaniasis continue to be a health hazard in Kenya in certain endemic areas. In the case of visceral leishmaniasis large areas of high population density are affected with small children and youth being the main victims. The disease is usually treated through administration of antimonial drugs for thirty days with daily injection of the drug. A second focus of cutaneous leishmaniasis disease is currently under investigation with six reported cases.

The work on Visceral leishmaniasis is centred mainly in Machakos District in Kibauni and Makueni Locations. The main areas of research are on the vector behaviour and animal reservoir.

Blood-meal analysis was conducted on species of sandflies captured from outdoor resting sites. This analysis was carried out to find habits of sandflies so as to narrow down the possible vector species of visceral leishmaniasis in Machakos. Dissections of parous females were conducted to investigate whether or not the insects were infected.

For studies of vector-behaviour, houses were sprayed with pyrethrum to determine the species resting indoor. A special trap was designed to facilitate collection of sandflies resting indoors and those flying into houses; a similar type was used for trapping around termite hills which are resting sites for sand-flies. Manbaited catches were made on both indoor and outdoor resting sites. A pilot control programme was carried out to assess the efficacy of DDT residual spray in the area.

Dogs were investigated for animal reservoir studies. Blood smears and cultures were made from spleen, liver, kidney and cardiac blood.

The blood-meal analysis from Machakos revealed that a majority of the sandflies species feed mainly on reptiles. Obsevations of animal activities in the resting sites revealed that lizards were numerous in these areas.

Table 4. **Numbers of** ***An. gambiae s.l.*** **collected between March and May 1979**

	JIMBO		JEGO	
	Total mosquitoes collected	Average per house	Total mosquitoes collected	Average per house
Males	5	0.1	22	0.5
Unfed females	13	0.3	25	0.5
Fed females	79	1.6	174	3.6
Semigravid females	12	0.3	75	1.6
Gravid females	0	0	28	0.6
Total females	104	2.3	302	6.3

Dissections of female flies revealed some promastigotes which were isolated in NNN medium and are still to be identified. One such isolated from *P. martini* an anthropophitic sandfly, turned out to be *Leishmania donovani* when analyzed by enzyme techniques and Excreted Factor Test.

The pilot control programme using DDT appeared quite effective for over a year with minimal vector re-infestation.

The animal reservoir studies so far have revealed three dogs infected with leishmaniasis. This may indicate that the dog is a domestic reservoir in this new epidemic zone.

The man-baited catches have revealed the presence of two major anthropophilic sandflies, namely: *P. martini* and *P. garnahami*. *P. bedfordi* role has been extensively studied while *P. garnhami* is currently under investigation for its role as a vector in this area.

GRASSLAND TERMITES RESEARCH PROGRAMME

Research Consultants
Professor C. Noirot
Dr. R. H. Leuthold

Programme Leader
Professor W. L. Nutting (1979)

Research Staff
Dr. M. A. Arshad (1977) Senior Research Scientist
Dr. J. P. E. Darlington (1975) Research Scientist
Dr. G. W. Oloo (1974) Research Scientist
Mr. B. M. Okot-Kotber (1976) Associate Scientific Officer
Dr. D. B. A. Ruyooka (1978) Postdoctoral Research Fellow
Dr. (Mrs.) V. D. P. Nair (1979) Postdoctoral Research Fellow
Dr. R. Sieber (1979) Postdoctoral Research Fellow
Dr. T. O. Oloya (1979) Postdoctoral Research Fellow
Miss M. G. Wanjiru (1978) Technical Assistant
Mr. E. Nyandat (1977) Technical Assistant
Mr. L. B. Busharizi (1977) Senior Technician
Mrs. M. N. Baraza (1974) Technician
Mr. M. A. Kotengo (1979) Junior Technician

The role of *Macrotermes* in soils

M. A. Arshad

The grass feeding termites remove considerable quantities of vegetation which might otherwise be utilized by grazing mammals. However, they also affect soil development and nutrient availability by transporting enormous amounts of soil and organic matter to mounds and other structures from which they are redistributed by erosion and decomposition. Termites also play a major role in plant productivity through influence on soil properties such as soil texture, structure, porosity, infiltration, water holding capacity and chemical characteristics. Any attempt to control or manage termite populations must therefore take into account their effects on soil productivity.

The present study was undertaken to determine the role of termites (*Macrotermes michaelseni*) in soil movement (gain or loss) and soil productivity in a semi-arid grassland ecosystem. It is being carried out about 8 km south east of Kajiado, Rift Valley Province, Kenya.

Effect on soil and water loss

Details of the experimental set up and methodology were outlined in the sixth ICIPE Annual Report (1978). Each of the six experimental plots (three with termites and three without termites) had a very dense cover of vegetation because of unusually high rains in 1977 and 1978. The first set of data on soil loss and run off (water loss) was recorded in 1979 and is shown in Table 1. Rainstorms with intensities less than 25 mm/hour were omitted. The run-off measured from each storm varied from less than 1 per cent to about 10 per cent of the total precipitation. No appreciable difference was found between the amounts of run-off from plots with active termite mounds and those with inactive mounds. The low yield of run-off is mainly due to the very dense vegetation in the experimental plots; which reduces run-off and soil loss and favours infiltration. The heaviest storm, on 3rd February, 1979, with an intensity of 120 mm/hr (maximum intensity recorded since the plots were set up) resulted in soil loss of 0.03 to 0.04 tons/ha. in various plots. No significant difference in the amount of soil loss occurred among the various treatments.

Effect on soil productivity

Vigorous and luxuriant growth of grasses forming a unique pattern in a green ring is commonly observed around the termite mounds and some possible reasons were given in the last ICIPE Annual Report. The dry matter yield in various plots was again estimated during 1979 and the data obtained confirmed the findings of the previous year. In order to investigate further the possible causes of growth variability, physical and chemical properties of soils in relation to the distance from the termite mounds were studied. The data are shown in Tables 2 and 3.

The bulk density of the top portion of the mound is quite high with a median value of 1.76 g/cm^3 but the density of the whole mound is relatively low ranging from 1.49 to 1.63. This is because of the numerous cavities in the nest system. The density at the base of the mound is low because of loosely deposited soil eroded from the mound. There is a general increase in soil density with gradual increase of distance from the mound.

The data on infiltration rates indicate that the permeability of the mound is very low and is attributable to the high compaction shown by its high bulk density. The maximum infiltration rate was found at a distance of 2 to 10 metres showing extremely rapid permeability in this region. This coincides with the maximum of

dry matter yield obtained in this region. This is attributable to numerous root channels and termite foraging holes observed in this area. Infiltration rates in the wet state (determined after 30 minutes of dry run) were considerably lower but showed a similar trend in relation to the proximity of the termite mound.

Moisture retention values at pF 2.3 and pF 3.7 ranged from 14.4 to 28.5% at various points with the higher moisture availability status between 1–10 metres from the mound. Percent available water at 0–1 metre is about the same as at 15-25 metres distance.

This relatively low water availability near the mound may partly be related to the drying-up of vegetation immediately around the mounds soon after the commencement of the dry season.

The data on chemical properties show a trend toward a slight decrease in pH values with increasing distance from the mound. However, the differences are small. Organic carbon and nitrogen values are low near the mound but higher amounts of these nutrients were found in the area of maximum vegetative matter. The ratios near the mounds appear to indicate a relatively high rate of mineralization as a result of termite activities.

Cation exchange capacities (CEC), exchangeable cations, and base saturation decrease with increasing distance from the mound (Table 3). This is directly related to clay contents and in some cases the organic matter. It is evident that the higher exchange status does have a favourable effect resulting in better grass growth around the mound soil.

Plant diversification

Luxuriant and vigorous vegetation observed around the termite mounds differs markedly in its composition from the species structure of the general study area (Table 4). The presence of such different plant species under similar climatic conditions must be attributed entirely to soil variability primarily caused by the termite acivities. Glover et al; (1964), found that, of the 59 species of plants identified on the Loita plains in Kenya, only 10 were recorded on termite mounds (*Odontotermes* sp.) and, of these, six appeared to be exclusive to the mounds. The percent distribution of species in relation to the termitaria was not studied by these workers. The variable distribution of grass species noted in the present investigation is attributed mainly to the differences in the edaphic characteristics such as texture, porosity, infiltration rates, water availability and soil chemical properties as discussed elsewhere in this report. Better drainage and deeper soil profile around the mound (which normally occur at higher elevations on the sloping landscape) than the surrounding soil may have also contributed to growth variability and species composition.

An interesting feature of the vegetation in the immediate vicinity of the termite mound is the absence of *Themeda triandra* and *Digitaria scalarum* which are major grass species occurring at 5–10 metres distance from the termite mound. Their percent stand then gradually increases until they blend with the remaining vegetation of the area at a distance of 20 metres. Major grass species of the termite mound are *Pennisetum stramineum* and *Cynodon dactylon*. The latter becomes a dominant species within the 1-10 metre zone (the green ring) around the termite mound and is totally absent beyond 15 metres distance down-slope. *Pennisetum mezianum* charateristically occurs at the base of the termite mound.

Another notable effect is the increase in the number of species with increasing distance from the termite mound. These results appear to indicate that termite activities, through their influence on soil conditions, tend to eliminate one vegetative cover and promote the development of another.

Table 1. Role of termites in soil and water loss (Kajiado, Kenya)

Date	Rainfall (mm)	Intensity (mm/hr)		Run-off (mm)		Soil loss (tons ha)	
		Max	Mean	T*	D**	T	D
29/1/79	14.4	31.0	18.2	0.18	0.17+	0.00+	0.00+
31/1/79	16.5	28.8	14.6	0.22	0.23	Tr.***	Tr.
1/2/79	14.2	44.0	26.0	2.50	2.75	0.01	0.01
3/2/79	97.0	120.0	80.0	8.50	9.00	0.04	0.03
19/2/79	18.0	26.0	16.1	2.10	2.25	Tr.	Tr.
21/2/79	27.7	26.0	23.0	2.50	2.45	Tr.	Tr.
22/2/79	36.5	52.0	32.0	3.80	3.91	0.01	0.01
18/3/79	44.2	50.0	36.5	1.85	1.99	0.00	0.00
20/3/79	32.0	27.0	21.5	1.50	1.35	0.00	0.00
10/4/79	44.8	40.0	21.5	0.50	0.55	0.00	0.00
8/5/79	18.5	28.0	13.5	0.20	0.10	0.00	0.00

*T: Plots with active termite mounds.
**D: Plots with inactive (dead) termite mounds.
***Tr: Traces—less than 0.005 tons/ha.
+: Sample mean of six replicates.

Table 2. Physical Properties of Soil in relation to the proximity of termite mounds (Kajiado, Kenya)

	Distance from mound	Texture Class	Bulk density	Infiltration		Moisture retention		Available water
				Dry	Wet	pF 2.3.	pF 3.7	(pF 2.3–pF 3.7)
⁻m⁻			g/cm³	⁻cm/hr⁻				
0	(Top of mound)	Clay	1.76+ (1.53)	2.0	1.3	29.2	23.1	6.1
	(Base of mound)	Clay	1.25	6.0	2.8	28.5	22.6	5.9
1		Sandy clay	1.28	23.6	12.0	24.5	18.1	6.4
2		Sandy clay loam	1.31	34.8	16.3	23.2	16.1	7.2
5		Sandy clay loam	1.30	47.5	22.3	22.5	14.6	7.9
10		Sandy clay loam	1.33	40.0	15.2	23.0	15.2	7.8
15		Sandy clay loam	1.37	18.6	8.7	20.4	14.4	6.0
20		Sandy clay loam	1.37	15.0	8.8	21.0	15.2	5.8
25		Sandy clay loam	1.39	12.6	8.0	20.5	14.5	6.0

+Median value of six replicates; the figure in brackets is the bulk density of the whole mound above ground.

Table 3. Chemical properties of soils in relation to the proximity of termite mounds (Kajiado, Kenya)

Distance	pH	Org. C	N	C/N	Exchangeable Cations				CEC	Base Saturation
					Ca	Mg	Na	K		
⁻m⁻		—%—					me/100g			%
0	5.6	0.91+	0.100	9.1	15.5	3.4	0.3	2.7	26.2	84
1	5.5	1.32	0.126	10.5	12.0	3.2	0.2	2.6	24.0	75
2	5.5	1.40	0.133	10.5	10.8	3.2	0.2	2.6	23.1	73
5	5.5	1.46	0.141	10.4	10.2	2.9	0.2	2.4	22.4	70
10	5.5	1.95	0.169	10.5	9.9	2.6	0.1	2.1	20.9	71
15	5.4	1.60	0.139	11.5	9.4	2.2	0.1	2.0	20.0	69
20	5.3	1.59	0.136	11.7	8.0	2.0	0.0	1.9	17.4	68
25	5.1	1.62	0.136	11.9	8.1	2.0	0.1	2.0	17.6	69

+All values are sample mean of duplicate determinations.

Table 4. Species structure of the vegetation in relation to the termite mounds (Kajiado, Kenya)

Species	Distance from mound (metres)						
	0–1	1–2	2–5	5–10	10–15	15–20	20–25
				Percent of stand			
Pennisetum mezianum Leeke	18	—	—	—	—	—	—
Pennisetum stramineum A. Peter	42	24	7	10	7	14	8
Cynodon dactylon (L) Pers.	40	76	93	72	27	—	—
Themeda triandra Forsk.	—	—	—	8	31	32	43
Digitaria scalarum Chiov.	—	—	—	—	15	40	32
Other species*	—	—	—	10	10	14	17
Total numberof species	3	2	2	4	6	7	6

All values are sample mean of three replicates.

**Chloris vox-burgiana* Trin., *Aristida keniensis* Henr., *Eustachys paspaloides* and *Eragrostis braunii. Schweinf*

TERMITE POPULATION ECOLOGY

J. P. E. C. Darlington

Macrotermes michaelseni—food storage structures

Lepage postulated the existence of temporary storage structures, undergound but outside the nest, in which food collected abcvc ground at night is stored until transported to the nest during the day (ICIPE Annual Report for 1977).

We have recently excavated a large segment of the underground foraging passages of a mature *M. michaelseni* nest in grassland near Kajiado. Large flat-floored passages 5 to 7 cm wide run out radially from the hive at a depth of 50 to 60 cm below soil level. At a distance of 5 to 10 metres from the nest the passages rise steeply to 10 to 25 cm below the surface, where they branch repeatedly and link up to form a dense anastomosing network of underground passages. These connect to the small foraging access holes which lead up to the surface.

Along the sides of the passages there are numerous elongated pits, usually 2 to 5 cm long and ½ to 1 cm wide, often grouped in threes around the junction with an access hole. In a few places fresh food has been found in the pits, so there can be no doubt that these are the temporary storage structures postulated by Lepage. Their individual volume is small, but there are about 10 to 14 pits per metre of passage, so that the total storage volume is quite substantial. This is the first time that such storage structures have been described outside a termite nest, although storage of food inside nests (which does not occur in *M. michaelseni* or *subhyalinus*) has been reported for *M. bellicosus* in West Africa and for *M. herus* in western Kenya, as well as in several other genera of termites.

A striking feature of the whole system is the high density of galleries under the soil and the great distance to which the network extends—about 50 m from the mound in this case. The combined effect of all these interconnected air spaces and access holes on the gross structure of the soil should be considerable.

Macrotermes subhyalinus—nest populations

Woik has continued on sampling the nest populations of *M. subhyalinus* at Bissel. We have data from nests ranging in total population from 381,000 to 2,024,000 sterile individuals. The series is not yet complete, and the progress of work has been slowed by some conspicuous failures in the fumigation technique on the open mounds of this species.

One such failure, in which a large dose of fumigant apparently failed to reach the hive or to kill any termites at all, provides an interesting comparison with an adjacent nest of similar size and condition in which the fumigation was successful. Data for the two nests are given in Table 5. Parameters of mound size, fungus weight and queen's weight are very similar; however, the total sterile population was more than seven times as great in the fumigated one as in the live-dug nest. The proportion of adults to larvae was similar in the two cases, but the caste ratios were quite different. The live-dug nest had proportionately more soldiers and major workers while the fumigated nest had more minor workers than any other caste. The disappearance of alates from live-dug nests (presumably into the extensive gallieries outside the nests) has often been observed in *M. michaelseni* also.

This comparison brings out the importance of fumigation in termite population assessments, particularly with massively constructed nests which take some time to dig up.

All previous population estimates for *Macrotermes* and similar genera have used data from live-dug nests, and are thus likely to have underestimated the populations by as much as an oider of magnitude.

The smallest *M. subhyalinus* nest so far sampled is of particular interest in that it showed far greater similarity in construction to *M. michaelseni* nests than those of mature *M. subhyalinus*. At this early stage the fungus combs are confined to the hive chamber where they are arranged in concentric shells around the central nursery, just as they are in young *M. michaelseni* colonies. However, the nest already has the open air passages characteristic of mature *M. subhyalinus* nests which have all the fungus combs in separate chambers outside the hive. We hope to find colonies of intermediate size showing the transition between the two arrangements.

Table 5. Comparison of live-dug and fumigated nests of *Macrotermes subhyalinus*

	Live-dug nest	Fumigated nest
Height of central mound, cm	95	80
Circumference of central mound, cm	1,215	950
Diameter of central mound, cm	391	345
Number of open air passages	38	27
Total dry weight of fungus combs, kg	21	19
Fresh weight of queen, g	21.5	21.9
Total population of sterile castes	267,400	1,909,000
Total number of adult steriles	197,706	1,360,000
(percent of total population)	(73 9%)	(71.3%)
Major soldiers (as percentage of total adult population)	6.5	1.8
Minor soldiers (as percentage of total adult population)	19.2	1.9
Major workers (as percentage of total adult population)	58.4	40.3
Minor workers (as percentage of total adult population)	16.0	56.1
Total number of alates	18	9,289

I. Behavioural and physiological changes in reproductives of *Trinervitermes bettonianus* (SJOST.) (Isoptera Termitidae) in the process of colony foundation

G. W. Oloo

In order to reproduce and establish new colonies successfully, the termite reproductives have to undergo the critical transition from emergence to swarming, pairing of mates, nest foundation, egg production, nursing of larvae until new workers take over this function and, finally, the attainment of physogastric stage. This study aimed to determine whether the organs functionally involved in the changing roles and behaviour of reproductives undergo morphological or physiological changes in development.

Materials and methods

The study material (from Machakos) consisted of weekly samples of preflight imagoes dug from field colonies, swarming imagoes, and reproductives from incipient laboratory colonies. The glands studied were the sternal gland (involved in courtship sequence, including tandem behaviour); the salivary gland (source of brood nutrition); ovary (reproduction) and corpora allata (possibly involved in the physiological and behavioural changes). The same females were dissected for all the glands studied. Observations on behaviour were confined to "tandem" that leads to nest foundation and "mouth-to-mouth contact" a typical feature of nursing.

To study tandem behaviour, a pair of imagoes was placed in a petri dish with moist soil; the number of tandem runs performed was recorded at 10 min. intervals over a 2 hour period; this was repeated with 5 pairs for each age group. For nursing activity, a pair of imagoes was placed with larvae from field colonies in a petri dish with moist soil; they were kept overnight in the insectary until the following day. The number of mouth-to-mouth contacts between male or female with larvae were recorded at 1 min. intervals over 1 hour period. For sternal gland activity, 10 female glands were dissected and extracted singly in 100µl hexane for 24 hours at 4°C; the extract was assayed for trail-following activity.

The condition of the ovary was determined from the total volume of developing oocytes; egg production was estimated from the total number of eggs present in 10 incipient colonies. The activity of the salivary gland was estimated from histological preparations by determining the relative cytoplasmic size reflected in the ratio of acinus cross-sectional area to the number of nuclei within the area. The corpora allata volume was determined from serial histological sections.

Results

The imagoes of *T. bettonianus* at Machakos normally emerge between middle and end of September in the dry season, but remain in the parental nest (for about 2 months) until the rains come, when they swarm. At emergence, the imagoes were gregarious in habit and showed no tendency to perform tandems; the disposition for tandem behaviour increased to a maximum at swarming stage. Newly emerged imagoes showed no motivation to nurse larvae; nursing activity was highest in the first cycle of brood production. The potential trail-laying activity of the sternal gland (expressed in "Trail Units", TU) increased sharply from 3,000 TU within 1–2 weeks after emergence (100TU recorded in newly moulted female alates) to 14,000TU in the swarming female, then declined rapidly after nest foundation (Fig. 1a). In the physogastric queen, extracts from the gland elicited no trail-following response. The ovary of freshly emerged females is undeveloped; it reached measurable size 4–5 weeks after emergence when a volume of about $2–3 \times 10^6 \mu m^3$ was recorded; this increased to $7 \times 10^6 \mu m^3$ at flight stage, then declined after the first cycle of egg production (Fig. 1b). Egg laying started 5–7 days after settling and was relatively high for the first 2 weeks, then declined and remained low even after several weeks of foraging (Fig. 1c). The relative size of the salivary gland increased to a maximum when the first offspring was produced (Fig. 1d). There was a general, but fluctuating, increase in corpora allata volume from emergence to swarming (Fig. 1e).

In the physogastric queen, a volume of about $20,000 \mu m^3$ was recorded as compared to $2,900 \mu m^3$ in the swarming female.

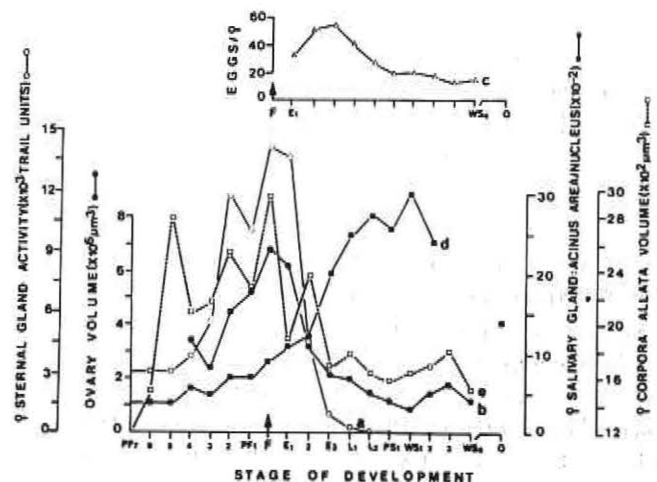


Figure 1. Behavioural and Physiological changes in *Trinervitermes bettonianus* (Sjost).

Discussion

The studies showed that newly emerged reproductives are not ready for swarming, pairing and reproductive functions before undergoing further development to attain full maturity. This partly explains the phenomenon of delayed flight that follows appearance of alates in the nest, previously attributed mainly to meteorological conditions. Development of the glands functionally involved in post-flight behaviour was correlated with the behavioural changes. There was a general correlation between changes in ovary and corpora allata development, suggesting a possible gonadotropic effect of juvenile hormone, which is well known in other insects. This report provides a descriptive background for experimental analysis of the relationship between the changing role and behaviour of reproductives with morphological and physiological changes in the glands involved.

II. Specificity of Termite Trails: Analysis with natural and extract trails of *Trinervitermes*, *Macrotermes* and *Odontotermes* from sympatric populations

Although it is well known that termites produce and utilize trail pheromones, the scope of their biological function in the ecological communities where the termites live is not fully understood. For the species under study, *Trinervitermes* and *Macrotermes* have similar pioneering foraging strategies, both forming open-air exploratory columns; the foraging range of *Macrotermes* and *Odontotermes* are extensive, with subterranean galleries spreading 20m or more from the nest. Such foraging behaviour raises the question: Can a stray termite discriminate between its own and other conspecific or alien trails of neighbouring colonies? In social Hymenoptera, there are several examples of non-specificity and varying degrees of trail specificity at species, genus, and sub-family levels. In termites, knowledge on trail specificity is fragmentary. This report examines the possibility of pheromone systems providing an isolation mechanism among members of sympatric termite communities.

Materials and Methods

The species studied were *Trinervitermes bettonianus* (Sjost.) *Macrotermes michaelseni* (Sjost.) and *Odontotermes* sp. (? *badius*) from Machakos. Whole nests of *T. bettonianus* were collected and maintained in the laboratory as previously described. To collect *M. michaelseni* a mound was opened up and a plastic basin filled with fresh fungus combs and nursery material placed in the main chamber to lure termites. *Odontotermes* were collected either from dung pads, on which they foraged, or from ventilation shafts, as they built them; the termites were shaken into a container having moist soil covered with moist filter paper.

The pair of colonies to be tested were connected to a food chamber by a bridge consisting of a "Y" test device with arms extending to the nests (Fig. 2a). The food chamber was partitioned with a thin wall extending to the junction of the "Y" tunnel, to separate foraging traffic closely enough for the 2 trails to be perceived simultaneously by a test termite. To compare natural trails food was offered; the termites deposited trails on a paper substrate placed beneath the "Y" tunnel; after 2 hours foraging, the paper was assayed for trail preferences (Fig. 2b) alternately with 20 single workers from each colony in a total of 40 runs. In further tests, equal numbers of trail-layers (30–100 workers) were used to minimize possible influence of trail strength. For extract trails, major worker sternites bearing the sternal gland were dissected and extracted (20 glands/ 200μl hexane) for 24 hours at 4°C. Extracts were applied (2.5μl/5cm) with a micropipette along either side of "Y" pencil line on fresh paper (Fig 2c) and tested alternately with 20 single workers from each colony; the trails were interchanged to minimize possible direction learning. All tests were repeated 4–7 times with different pairs of colonies.

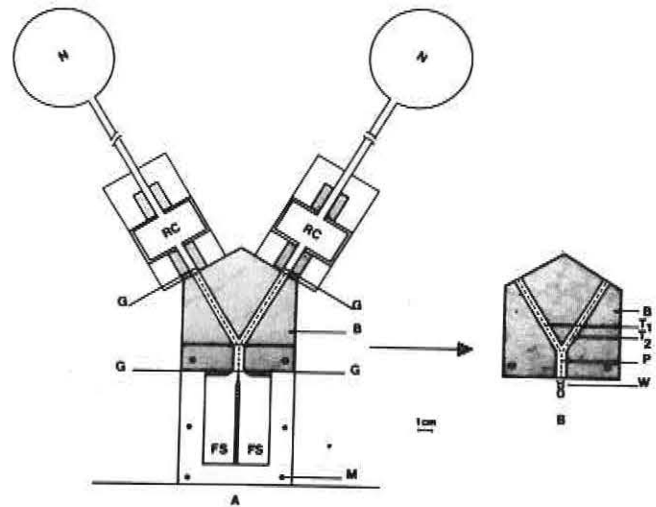


Figure 2. The set-up for assaying trails for specificity.

Results

Analysis for selective trail-following with well established natural trails indicate that termites from sympatric colonies of *T. bettonianus* do not show a significant preference for their own colony's trails in a choice situation (Table 6). However, some preferences were recorded even when equal numbers of trail-layers or glands were used. On the other hand, sympatric

populations of *T. bettonianus*, *M. michaelseni* and *Odontotermes* sp. recognize their respective trails with a high degree of precision (Tables 7 and 8).

Discussion

The present studies indicate that workers of *T. bettonianus* do not show a significant preference for their own colony's trails; the recorded cases of preferential trail-following may be attributed to differences in trail-laying capacity from one colony to another, on the basis of experiments where equal numbers of workers or glands were used to generate trails. These results imply that a stray termite could end up in the wrong colony. However, field observations indicate the existence of adequate Isolation mechanisms during foraging, including colony odour, orderly guarding of foraging routes by soldiers, the tendency to forage close to the nest out of range of neighbouring colonies, etc. On the other hand, evidence from these laboratory experiments strongly suggest that *Trinervitermes*, *Macrotermes* and *Odontotermes*, employ different pheromonal systems in the co-ordination of their foraging activities and thus provide an isolation mechanism among members of sympatric communities of these termites.

Table 6. Selective trail-following: Analysis with established natural trails of Sympatric Colonies (Ma and Mb) of *T. bettonianus* from Machakos

Source of trail and test termite	Trail-following response+ Replicates (colony pairs tested) I	II	III	IV	V
Ma	14	12	5(*)	19**	2(**)
Mb	7	7	13	0(**)	18**

+, No. positive choices of own colony's trail (out of 20 choices between own and neighbouring colony's trail).
*, significant at $p<0.05$; **, significant at $p<0.01$; (**), significant preference for alien trail.

Table 7. Specificity of pheromonal trails: Analysis with established natural trails of sympatric colonies of *T. bettonianus* (Tb), *M. michaelseni* (Mm) and *Odontotermes* sp. (o) from Machakos—

Source of trail and test termite	Trail following response+ Replicates (colony pairs tested) I	II	III	IV	V	VI	VII
Tb	20**	20**	20**	20**	18**	20**	20**
Mm	19**	20**	16**	20**	20**	20**	20**
Tb	20**	19**	20**	20**	20**		
O	15*	19**	20**	20**	20**		
Mm	19**	16*	20**	20**	20**		
O	19**	20**	20**	15*	20**		

+, No. positive choices of own colony's trail (out of 20 choices between own and other species trail) significant at $p<0.05$ (*) or $p<0.01$ (**).

Table 8. Specificity of pheromonal trails: Analysis with extract trails prepared from equal no. major worker sternal glands of *T. bettonianus* (Tb) *M. michaelseni* (mm) and *Odontotermes* sp. (O) from Machakos

Source of trail and test termite	Trail-following response+ Replicates (colony pairs tested) I	II	III	IV	V
Tb_1	14	12	15*	12	
Tb_2	11	9	13	14	
Tb	20**	16*	20**	20**	18**
Mm	20**	20**	20**	20**	20**
Tb	20**	20**	20**	17**	20**
O	20**	20**	20**	19**	20**
Mm	20**	20**	20**	20**	
O	20**	15*	17**	20**	

+, *, **, (**) (See Tables 5 and 6 for explanation).

Juvenile hormones and caste differentiation in *Macrotermes michaelseni* (Isoptera, Macrotermitinae)

B. M. Okot-Kotber

Previous experiments have implicated the endocrine glands in the differentiation of soldiers and reproductives. Two studies were therefore undertaken to determine the role of juvenile hormone (JH) in the differentiation of larvae to presoldiers: One to determine the competence of larvae to differentiate, the other to compare the effects of the method of hormone application on rates of presoldier formation and the types of soldiers so formed. Groups of third instar larvae from laboratory colonies were used. Juvenile hormone analogue (JHA), ZR-515, was applied either topically or as vapour to the experimental larvae to mimic juvenile hormone action.

The influence of topically applied JHA on male and female larvae

Earlier studies have shown that soldiers develop only from female third instar larvae. Here, an attempt was made to induce soldier formation from third instar male larvae as well. The experimental animals were topically treated with 2.0μg JHA in 0.5μl redistilled acetone and adopted by pairs of reproductives. The development of treated individuals was followed closely and recorded every other day.

The survival rates of treated and untreated individuals were good (60–80%) and comparable. In control groups of female larvae, soldier formation rate was about 7–10% while in treated groups it was about 26% (Fig. 3a and b). On the other hand, all untreated male larvae (controls) developed into major workers while about 18% of JHA-treated ones developed into soldiers (Fig. 4a and b). Some of the treated male and female

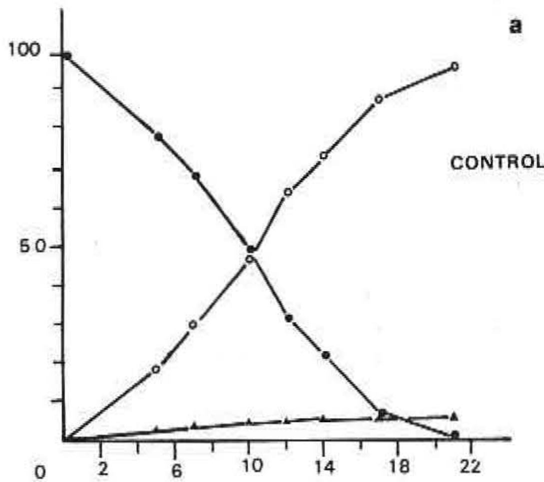


Figure 3a. Rate of presoldier formation in contrcl colonies which had adopted third instar female larvae.

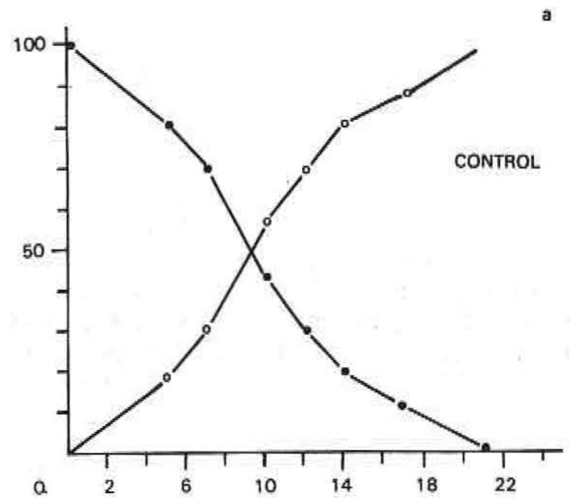


Figure 4a. The developmental trend in control colonies containing adopted male third instar larvae.

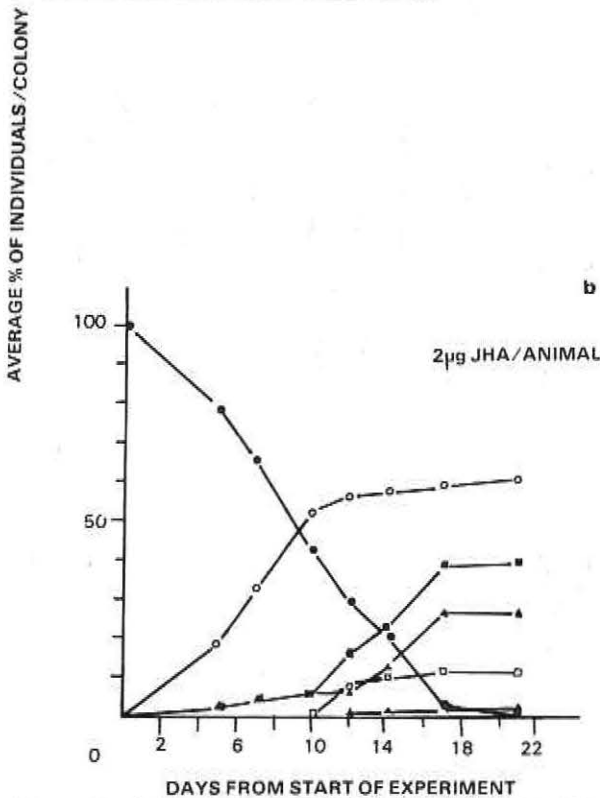


Figure 3b. Rate of presoldier formation and other options in topically JHA treated third instar female larvae. Workers (0). Larvae (●). Presoldiers (▲), Presoldier-like (□), Worker-like (Δ). total of affected individual (■).

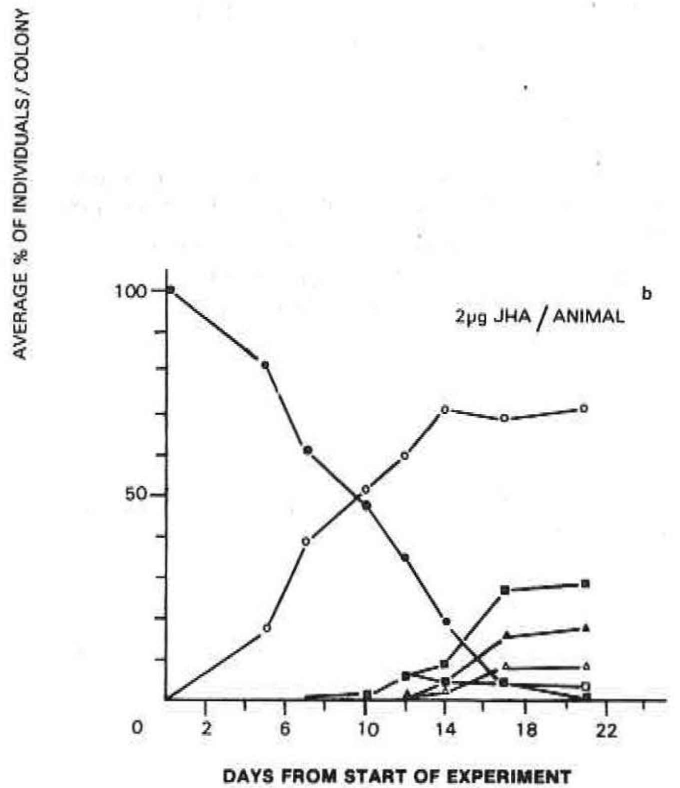
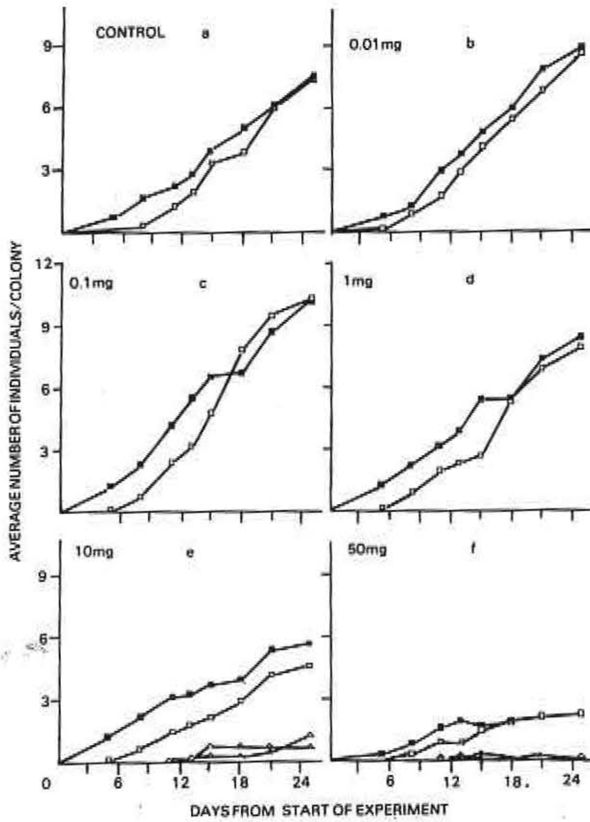
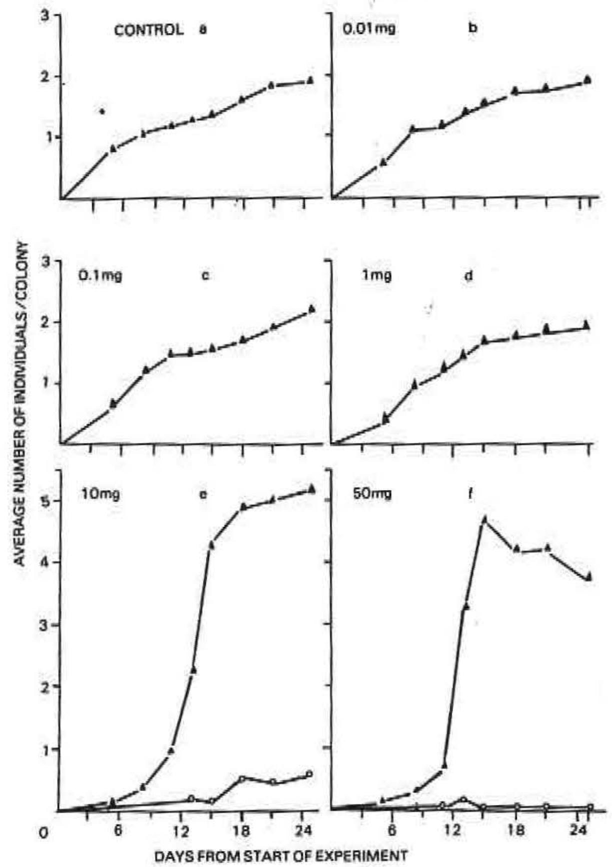


Figure 4b. Rates of presoldier (▲), presoldier-like (Δ), worker-like (□) and worker (0) formation in adopted third instar male larvae topically treated with JHA (●),. Total affected individual (■).



Figures 5a, b, c, d, e and f. Rates of minor (■) and major (□) worker replacement in incipient colonies where workers, presoldiers had been removed and the colonies treated with different levels of JHA vapour. Note that worker-like individuals (▲), minor worker-like (Δ), major worker-like were also formed under 10–50 mg. JHA vapour.



Figures 6a, b, c, d, e and f. Rate and level of presoldier replacement in incipient colonies under the influence of various levels of JHA vapour. Presoldiers (▲) and Presoldier-like (0).

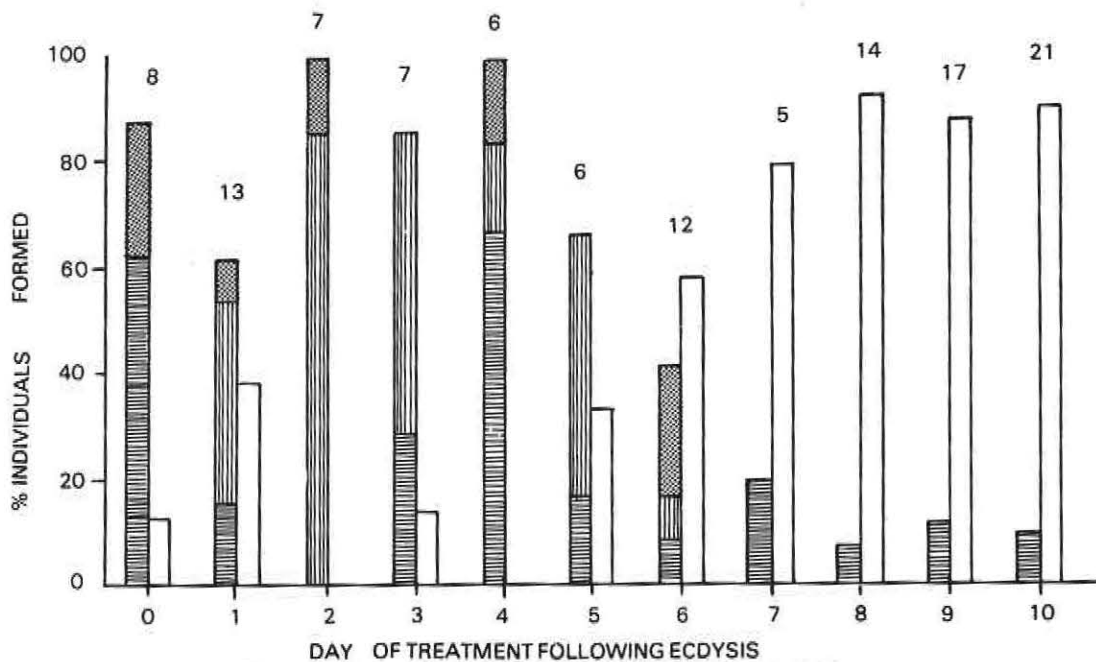


Figure 7. Formation rate of presoldiers and other individuals from larvae of different ages topically treated with JHA ( ) presoldiers ( ), presoldier-like (□), workers ( ) worker-like. Figures above the columns represent the total number of individuals observed.

larvae developed into intermediate forms (intercastes) but the overall percentage of affected males was smaller (30%) compared to 38% of female larvae.

Biometric studies of male and female presoldiers and soldiers formed under JHA treatment showed that males are larger than females (larger head capsules and longer mandibles) with the exception of mandibular index (ratio between head capsule length and mandibular length) which was comparable. However, mandibular index of the two types of JHA-produced presoldiers was smaller ($P < 0.05$) than that of normally produced presoldiers (presoldiers from control groups).

Effects of prolonged JHA vapour exposure on developing incipient colonies

Colonies used in this experiment were deprived of their presoldiers/soldiers, minor and major workers, leaving behind only larvae of different instars and sexes. Varying doses of JHA were used (0.01, 0.1, 1.0, 10.0 and 50.0mg/colony) and allowed to vaporize from drops applied to the petri dish covers.

High mortality occurred in colonies treated with 50 mg JHA, with both reproductives and larvae dying in most of them. Workers were replaced at a lower rate in colonies treated with more than 1.0mg JHA (Fig. 5 a–f) while presoldier replacement was accelerated in the same colonies (Fig. 6 a–f). An average of 5 presoldiers per colony appeared in colonies treated with 10–50 mg JHA while controls or those treated with 0.01–1 mg had only 2 per colony. Presoldier-like individuals were formed in the colonies responding to JHA as was observed during topical treatment. The per-centages of each type of individual formed are summarized in Table 9.

Biometric studies showed that male and female presoldiers/soldiers were also formed under JHA-vapour treatment. Female presoldiers/soldiers were smaller than males produced under the same conditions of JHA treatment; furthermore, these females were smaller than those produced in the control groups. Mandibular index of male presoldier was smaller than in the control presoldiers ($P < 0.01$). However, this index was comparable in the two types of soldiers induced by JHA vapour.

Competence of larvae to differentiate into soldiers under the influence of JHA

Homogeneous groups of female third instar larvae were collected from donor colonies each day for 10 days. On the tenth day they were all topically treated with 2.0mg JHA, observed daily thereafter, and the type of moult which ensued was recorded. The results of this study are summarized in Figure 7. The individuals which responded moulted into presoldiers and the array of intermediate forms described above. The rate of formation of presoldiers and presoldier intermediates was high when larvae five days old or less were treated. Six-day-old larvae also responded to the treatment, but at a relatively lower rate (Fig. 7). No response was shown by larvae 7 days old or more.

These results have shown that, although topical treatment of larvae with JHA induces soldier formation in both sexes, the rates are lower than when these individuals are exposed to JHA vapour. In most cases more normal presoldiers and fewer intermediates are formed under vapour treatment. This suggests that a continuous supply of JH is required for normal soldier formation even after differentiation has occurred.

The finding that soldier formation can be induced by JHA in male as in female larvae is, in itself, very interesting because it strongly suggests that male and female larvae are genetically identical with respect to soldierforming potential. The difference seems to be only in the sensitivity of the genetic apparatus in responding to external influence.

The sensitive period (competence period) for soldier formation has been established: Third instar larvae are competent to differentiate into soldiers only during the first half of the instar after which worker development invaliably ensues. Further studies are designed to follow closely the events that may lead to soldier idfferentiation, particularly hormonal interplay.

Table 9. A summary of different individuals produced in incipient colonies following JHA vapour exposure

JHA DOSE Mg/colony	Minor workers %	Major workers %	Minor workers intermediates %	Major worker intermediates %	Presoldiers %	Presoldiers intermediate %
Control	45	40	0	0	15	0
10	32	18	3	5	40	2
50	20	18	2	6	53	1

Studies on caste ratio regulation in incipient colonies of *Macrotermes michaelseni*.

D. B. Abooki Ruyooka

In studies of caste ratio regulation, Lüscher, Springhetti and others have demonstrated in lower termites that the presence of soldiers in laboratory incipient colonies inhibits development of "competent" larvae into further soldiers. Curiously, these soldiers stimulate the larvae to moult into neotenic reproductives. On the other hand, the reproductives stimulate larvae to moult into soldiers. The existence of such an influence (a pheromone?) in higher termites has not been convincingly demonstrated. This report documents results of preliminary experiments with *Macrotermes michaelseni* (Sjöstedt) (Isoptera: Termitidae).

Six fractions of an extract (from Professor W. S. Bowers, Cornell University) of minor *M. michaelseni* soldiers were each thoroughly mixed with mixed mound soil of the same species. Each fraction was used in concentrations of 100% and 10% (in methylene chloride) as substrate for the colonies in 6-cm dia plastic petri dishes. The colonies each consisted of a varying number of larvae and two reproductives. The experiment was replicated ten times. The colonies were held at 29±1°C for 26 days, during which period they were inspected daily and any minor soldiers removed.

The results indicated that the extract had not affected any detectable influence on the differentiation of soldiers. This might be due to several reasons. If an inhibitory substance does, indeed, exist, it may have volatilised or otherwise been weakened or lost from the extract. The method of administration (bioassay) may somehow have been inappropriate. On the other hand, inhibition may be affected through some other mode, or not by chemical means at all.

Search for the mode or source of inhibition is continuing in this species, by testing the action of living soldiers on early third instar larvae.

Optimum temperature for development of *M. michaelseni*

As our experimental work depends heavily on the successful laboratory culture of incipient colonies, it is of utmost importance that they be maintained at optimum temperature for growth and development. A series of experiments toward this end were set up as follows.

Reproductives were collected in Kajiado town (Rift Valley Province), reared and set up in the usual manner (see ICIPE 1977 Annual Report, p. 31) in unsterilised mound soil. They were maintained in three incubators, each preset at 26°C, 29°C and 32°C for 90, 120, 150 and 180 days and checked daily. In all colonies (10 replicates), a small piece of fungus comb was added soon after the first workers began foraging, about two months after colony establishment.

Overall, the results showed that the optimum temperature was 29±1°C, judging from the number and physical nature of termites present in the containers. After 90 days 26°C, the colonies predominantly consisted of larvae (Figure 8).

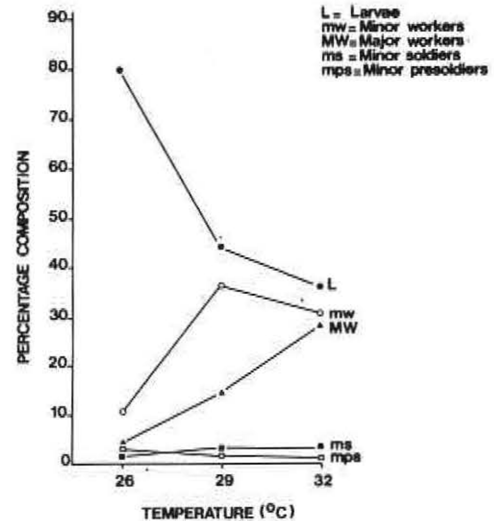


Figure 8 Percentage composition of incipient colonies after 90 days (average of 10 replicates).

The proportions of each caste and larvae remained fairly constant at each temperature over a period of 90 days. At 32°C, there were no soldiers or presoldiers after 150 days and all the brood and reproductives were dead after 180 days. That the percentage of larvae after 90 days at 32°C was 80% of the total brood is interesting, but it is not exactly known why this percentage is not maintained thereafter.

Food handling and fungus comb turnover in laboratory and field *Macrotermes michaelseni* colonies

M. michaelseni forages mainly on grass litter but, in adverse conditions, may actually consume standing grass and even roots. Although it is known that grass is the basic source of energy for this termite, it is still not clear how it is incorporated into the fungus comb on which the workers finally feed. That the fungus of the genus *Termitomyces* Heim is important in food digestion has been mentioned by a number of workers (e.g. W. A. Sands); the fungus provides digestive enzymes. Fungus comb dynamics demand careful study, including growth, cropping and turnover rate.

Work using laboratory incipient colonies is in progress to test various coloured dyes (detectable in minute quantities under UV light) for staining the food (fungus comb or grass). The passage of the food is then followed through the gut up to the time the semi-liquid pellets are deposited on the existing fungus comb.

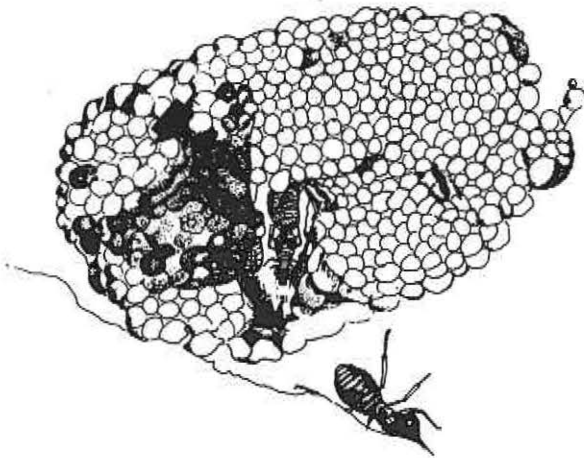


Figure 9 Deposition of faecal pellets coloured by a calco red dye on the surface of an existing fungus comb.

Blended fungus comb mixed with a 0.2% calco red dye took eight hours to reach the rectum and be deposited on the upper surface of the comb (Figure 9). During that period, grass boiled in 1% methylene blue and eaten by similar colonies had only reached the paunch. The slow progress of the latter food through the gut suggests that, rough as it is, grass cannot be so quickly and efficiently processed as fungus comb.

Predation of grassland termites in the semi-arid savannah at Kajiado

G. H. N. Nyamasyo

The role of organisms in a community is closely tied to their feeding relationships. Termites (Isoptera) are herbivores and above them in the food chain of living organisms are to be found predators or parasites. Predation is thus an important subject in the ecology of grassland termites. In March, 1979, a project on the predation of grassland termites was initiated at Kajiado, the purpose of which is to identify the major predatory species and to assess the offtake of termites in a semi-arid savannah ecosystem.

Ants (Hymenoptera: Formicidae) have obvious ecological interactions with termites particularly in the aspect of predator-prey relationships and were chosen as a starting point, although certain species of lizards, snakes, birds and mammals are also important termite predators.

The ant fauna of Kajiado

Specimens of ants collected at Kajiado were identified by Mr. B. Bolton of the Commonwealth Institute of Entomology, British Museum (Natural History). The species, their relative abundance ($3200m^2$ surveyed) and their observed feeding behaviour at Kajiado are given in Table 9. The list is not exhaustive but provides a good basis for predation studies. The nest densities reflected the situation at the study area between June and October.

The composition of animal food taken by ants

A total of 5439 animal food items were taken between June and November by various species of ants from a belt transect of 20×500 m. Initially the sampling was done daily at two-hour intervals but later reduced to one day in a week. The composition of the food items is presented in Table 10. Not all of these were preyed upon, some were simply scavenged dead. Since the feeding by ants takes place inside the nests it was assumed that the food items would meet the nutritional requirements of either the adult ants or their larvae.

The animal food preferences of ants

The list of animal food items was further analysed to give an idea of the food preferences of the ants and this is presented in Table 11. Two important predatory ants outside this table are *M. foetens* and *P. krugeri.* The former is an obligate predator of foraging termites and could not be studied along with the others because of its specialised hunting behaviour (see Lepage, ICIPE Annual Report, 1978) while the latter has not been thoroughly studied since it is active only at night.

The fact that over 29% of the food taken by ants at Kajiado is composed of termites shows how important a link the latter are in the food chain; that over 67% of their food is composed of other ants also demonstrates the complexity of the ants' food web. It should be noted that the termites referred to here are the foraging workers and not the alates. Since this project is still in its initial stages it is not yet possible to point out the significance of termite predation by ants. However, *M. foetens* has emerged as an important predator of *Macrotermes* which is perhaps the most important termite species in the area. Much more effort will therefore be directed towards studying *M. foetens* in the future.

Table 9. Ant species and their relative abundance at Kajiado

Ant Species	Estimated density per Ha.	Feeding behaviour-
Megaponera foetens F.	5	Obligate termite predator
Opthalmopone berthoudi F.	5	General insectivore/ carnivore
Playthyrea cribrinodis Gerstaecker	45	,,
Pachycondyla krugeri F.	55	,,
Dorylus sp A	10	,,
Dorylus sp B	—	,,
Camponotus sp A (maculatus groups)	160	,,
Camponotus nr. flavomarginatus (rufoglaucus groups)	20	,,
Camponotus sp B (maculatus groups)	60	Plant feeder
Messor cephalotes E.	5	,,
Ocymrmex weitzeckeri E.	25	,,
Messor sp B.	5	,,?
Pheidole sp	6	Catholic feeder
Tetramorium sericeiventre E.	30	?
Meranoplus simoni E.	—	?

Table 10. The composition of animal food items taken by ants during the period June—November, 1979

Food items	Percentage of toal	Food item	Percentage of total
Unidentified ants	34.7	Unidentified arthropods	3.5
Pheidole sp	19.6	*Macrotermes sp.*	2.7
Odontotermes sp	11.4	*Messor sp B*	2.1
Hodotermes sp	6.3	*Componotus nr. flavomarginatus*	1.9
Trinerviternes sp	5.1	*M. foetens*	0.5
T. sericeiventre	4.0	*Camponotus sp B.*	0.5
Microtermes sp	3.9	*P. cribrinodis*	0.1
Dorylus sp A	3.9	*O. weitzeckeri*	0.1

Table 11. The food preference of some species of ants

Ant species (predator)	Prey, % total Termites	Ants	Anthropods	Total
Pheidole sp	17.7	27.5	1.0	46.2
T. sericeiventre	3.4	34.2	1.0	38.6
P. cribrinodis	3.7	3.2	0.9	7.9
O. berthoudi	3.3	0.0	0.0	3.3
Messor sp B	0.5	1.4	0.1	2.0
O. weitzeckeri	0.3	0.9	0.3	1.5
Camponotus sp A	0.2	0.1	0.1	0.4
Camponotus nr. flavomarginatus	0.2	0.0	0.0	0.2
Total, %	29.3	67.5	3.5	100.0

TSETSE RESEARCH
REPRODUCTIVE PHYSIOLOGY

Director of Research
Professor T. R. Odhiambo (1970)

Research Staff
Dr. M. F. B. Chaudhry (1974) Senior Research Scientist
Mr. T. S. Dhadialla (1973) Scientific Officer
Mrs R. W. Kunyiha (1976) Research Assistant
Mr. F. Mukunza (1973) Junior Technician
Mr P. Osula (1978) Junior Technician
Dr. M. S. Ramasamy (1979) Research Scientist

Introduction

Tsetse flies reproduce by adenotrophic viviparity, giving birth, at regular intervals, to single fully developed larva. Following larviposition the maggot pupariates almost immediately without further feeding. Cyclical events of tsetse reproduction such as egg maturation, ovulation, larval development, secretory activity of the uterine glands (which supply nourishment to the developing larva) and parturition are regulated with marked accuracy by the female tsetse fly. The combination of the low rate of fecundity and the complexities of the reproductive process can be considered a vulnerable feature of the tsetse physiology.

The objective of the research was to study various events of the tsetse reproductive process in order to understand the underlying mechanisms which regulate those complex events and employ the knowledge to disrupt the pregnancy cycle. To achieve this, several research projects were undertaken during the course of the year. Results of these investigations are presented here under separate headings.

Development of the Female Reproductive and Endocrine Organs of the Tsetse Fly. *Glossina morsitans morsitans*

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

The objective of the research was to study the development of various reproductive organs and neuroendocrine tissues in embryonic, larval and puparial stages in order to provide information for exploring the controlling factors involved in the developmental processes.

Conventional histological techniques were employed to study the reproductive and endocrine organs in various stages of embryo, larva and puparium.

The first indication of the reproductive organ was noticed in a 72-hour-old embryo. The paired organ, located in the posterior third of the embryo, consists of a group of cells which bud off from the mesoderm. This mass of tissue increases in size following hatching and during most of the developmental period of the larva, but does not undergo any morphogenesis. Reproductive organs such as ovaries and spermathecae are not recognizable in the intra-uterine larva. The female reproductive system of the newly deposited larva is, thus, represented only by a pair of multicellular organ (described above) in the abdominal segments apparently terminating in a duct. This organ later develops into the uterine milk gland during early puparial life. No other reproductive organs are recognizable in the newly deposited larva.

A mass of tissue representing uterine wall is differentiated by the third day of puparial life. The uterus appears as a thickening and invagination of the body wall and its inner lining is continuous with the cuticle.

By day 8 of the puparial life, the two ducts of the uterine glands join into a common duct which opens into the uterus. At this stage the uterine cavity has enlarged, but the duct connecting the future milk gland with the uterus is extremely short.

In 9-day-old puparium, the developing milk gland forms several branches where lumens are evident. At this stage the milk gland cells are small and irregular in shape.

In 11-day-old puparium, ovaries are recognizable as a paired structure with paired equal sized ovarioles enclosed in a common sheath on either side. Germaria of ovarioles are recognizable at this stage. The ovaries have their ducts leading into the uterus. At this stage of the puparial life the milk gland has remified to some extent and the distal ends of the various tubules continue to divide and proliferate.

The opening of the common oviduct and the milk gland can easily be distinguished in sections from 12-day-old puparium. In addition, a third duct opening in the uterus appears at this stage. This opening is for the spermathecal duct.

The spermathecae and their ducts are recognizable in the sections of 13-day-old puparium. The two ducts are separate from one another. In 13 to 16-day-old puparia, most of the differentiated organs grow in size, the milk gland being remified into the lower abdominal space.

By the 18th day of the puparial life, the uterus has enlarged and its muscles have become relatively thinner. All the ducts opening into the uterus seem to be well developed and have by now moved some what to the back of the uterus. Numerous folding of the uterine wall occurs during this period and the 20th day of the puparial life.

By the 25th day the female reproductive system is almost fully developed. The development of the ovary has taken place showing trophocytes and the oocyte for the first follicle. In addition, there is evidence of yolk deposition in the first follicle.

An invagination appears at the anterior end of a 3-day-old embryo and some ecdodermal cells bud off and begin migrating posteriorly. These cells form the first tissue of the central nervous system. A foramen is formed in this tissue during this time through which the gut passes. The cells differentiate into neuroblasts and sink deeper into the mesoderm. Eventually they form a 3-lobed structure—the two lateral lobes and one median lobe which constitute the brain. Another group of cells form a central mass dorsally and these later develop into corpus allatum (CA) and corpus cardiacum (CC).

In a newly deposited larva the CA and CC cells have differentiated. The CA cells appear small and arranged in an oval mass, but the cells are not well demarcated. The CC cells are relatively large, loosely packed and with large nuclei. These cells lie lateral to and beneath the CA cells. No neurosecretory cells are recognized during the larval stage.

In a 3-day-old puparium, some neurosecretory cells are visible in the brain. Six days after pupariation, evagination of the head is apparent and there is a gradual increase in the length of the ventral nerve cord. Meanwhile the CACC complex begins to move away from the cerebral complex. In an 8-day-old puparium, the arrangement of the CA, CC and the aorta resembles that of the teneral fly. Although neurosecretory cells are present during most of the puparial life, results of the PF staining indicate that the cells are inactive during puparial life as well as in the newly emerged females.

Oocyte Development in *Glossina morsitans morsitans*: Vitellogenesis

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

Vitellogenin, the female specific protein in insects, is synthesized by the fat body of the female and is then transported via the haemolymph to the oocyte surface where it is taken up by micropinocytosis. In *G. m. morsitans* the process of vitellogenesis and yolk incorporation begins before the eclosion of the adult (see preceding section of this Report). The importance of the corpus allatum (CA) hormone in activating the fat body to synthesize vitellogenin has been demonstrated in other insects. In the tsetse fly, the CA hormone involvement in the vitellogenesis and yolk incorporation process is not as obvious as in the other insects, although the hormone seems to be required for the synthesis and/or incorporation (ICIPE Annual Report, 1978).

The objective of the present study was to determine the role of fat body and haemolymph in vitellogenesis of the tsetse fly, *G. m. morsitans*. In order to fulfill this objective, our initial attempts were concentrated on, (a) collecting tsetse eggs (a tedious undertaking since a female tsetse develops only one egg at a time over a 9-day pregnancy cycle). (b) separating and purifying vitellin proteins from the collected eggs, (c) raising antibodies to the isolated vitellins, and (d) to scan various tsetse tissues to determine sources of vitellogenin.

Protein fractions were separated by the method of E. C. Mundal and J. H. Law, University of Chicago, Chicago, U.S.A. (personal communication) which uses a Sepharose 6 B column at 4°C. Two of the protein fractions separated were indidivually concentrated by pressure dialysis. The protein concentration in the two fractions (hereafter referred to as A and B) was determined spectrophotometrically. Two rabbits were immunized, one with antigen A and Freund's Complete Adjuvant (1:1, v/v) and the other with antigen B and FCA (1:1, v/v), both of which were given booster injections two weeks later with the respective antigen and Freund's Incomplete Adjuvant in the same proportion as above. The rabbits were bled two weeks later and the antisera were collected. Ouchterloney's immunodiffusion technique was used to obtain various immunological reactions.

Antigen A gave the best reaction with antisera A when the former was used at 1:4, 1:8 and 1:16 dilutions. Antisera B also reacted with antigen B to give a faint precipitation arc. Antisera A showed cross-reactivity with antigen B.

The two antisera were allowed to react with samples of egg homogenate, homogenates of male and female 27-day-old puparia and haemolymph. Precipitation arcs showing complete identity of antigens were obtained when antisera B was reacted with homogenates of egg and female puparium. However, antisera B also reacted with the homogenate of male puparium. There was no reaction between the antisera B and the fat body homgenate or haemolymph from old virgin females (37-day-old).

Antisera to antigen A showed a very strong cross-reaction with egg homogenates: in fact, two precipitation arcs were obtained in this reaction. While this antisera did not react with fat body homogenates or the haemolymph from 37-day-old virgin females, it reacted with haemolymph from 3-day-old virgin females as well as with male haemolymph.

These results indicate that the antisera to the two antigens are not mono-specific. While at this stage of the study it is difficult to confirm the presence of a female specific vitellogenic protein in the fat body

or haemolymph, additional experiments need to be conducted to determine more precisely the nature and origin of the protein components and the mechanisms of transport of the yolk protein in tsetse flies.

We have concurrently conducted experiments using polyacrylamide disc gel electrophoresis and pH gradient electrophoresis on ampholine plates (pH range 3.5-9.0) to determine overall protein in the haemolymph of male and female and the egg homogenate.

Results of the disc gel electrophoresis of haemolymph showed that the protein patterns of the male and female haemolymph were identical. Eighteen protein bands were separated from each of the haemolymph, six of which are major protein bands (indicated by staining intensity). The intensely stained bands were located in the fast moving protein region. Although the resolution and separation were improved by using pH gradient electro phoresis (44 protein bands), the protein patterns of both male and female haemolymph were identical.

Tsetse eggs were homogenized in phosphate buffer (pH 7) and the homogenate was subjected to centrifugation at 10,000 rpm at 4°C for 20 min. The supernatent was used for electrophoresis.

Once again, more proteins were separated by the pH gradient electrophoresis than by the disc gel method. The protein pattern of the egg homogenate extract is different from that of male and female haemolymph protein patterns. Electrophoresis of the egg extract on ampholine plates revealed 38 protein bands of which 6 stained intensely. While few of the bands from the egg extract appear to have similar electrophoretic mobilities to some of the protein bands from the male and female haemolymph, it is difficult to say from these results alone if they are also antigenically similar proteins.

Regulation of Ovulation in *Glossina morsitans morsitans*

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

The female tsetse fly will ovulate only after she has successfully mated. Results of our earlier experiments suggest (ICIPE Annual Report, 1975 and 1976, Chaudhury and Dhadialla 1976) that the act of mating triggers ovulation by stimulating the female to release a neurohormone from the brain that is conveyed by the haemolymph to its target organ, the ovary. Subsequently, we have shown that ovulation can be induced in virgin mature females with dibutyryl cyclic AMP, cholera toxin (a cAMP generator) or a phosphodiesterase inhibitor (ICIPE Annual Report, 1978, Denlinger *et al.* 1978). We suggested that cAMP exerts its effect by stimulating the release of the ovulation-stimulating factor from the brain. Alternatively, it is more likely that the ovulation-stimulating hormone, like many other neurohormones uses cAMP as a second messenger in triggering its response within the ovary.

Additionally, our earlier results indicate that the appearance of the ovulation-stimulating hormone (OSH) in the haemolymph is not only dependent on the female's mating status but also on the stage of ovarian development (ICIPE Annual Report, 1976).

Experiments were conducted to investigate the possible existence of a second factor originating from a mature follicle which may also be responsible for controlling ovulation process.

In the first series of experiments, homogenate of mature eggs from mated females was injected into virgin mature females which had in addition to ovulating donor's haemolymph, virgin's haemolymph or neither.

In a second series of experiments mature virgin recipients were injected with the haemolymph from the following categories of donors;

(a) Virgins with fully-developed egg,
(b) Virgins with under-developed eggs,
(c) Mated females with fully-developed egg,
(d) Mated females with under-developed eggs, and
(e) Mated and ovariectomized females.

In the first series, ovulation resulted in 65% recipients when ovulating donor's haemolymph and egg homogenate were injected. Seventy-three per cent of the control recipients injected with only ovulating donor's haemolymph also ovulated. Injection of egg homogenate with virgin's haemolymph or egg homogenate alone did not result in ovulation of the recipients.

In the second series, none of the recipients ovulated except 75% of the females, which received injections of haemolymph donated by mated females with fully-developed egg.

Involvement of a second hormone from the ovary (in addition to OSH from the brain) in inducing ovulation cannot be completely ruled out, however, it is more likely that the ovary with a mature follicle regulates the release and/or transport of the OSH from the cerebral neurosecretory cells. The transported material accumulates in the neurohemal organ, the cervical portion of the aorta, until the female mates when the accumulated material is then released into the circulation.

The female *G. m. morsitans* generally mates when 2 to 3 days old but does not ovulate until another 7–8 days when the first follicle is fully-developed. The fact that ovulation occurs within 20 hours of the termination of the copulation in delayed mated individuals (Chaudhury, 1977) indicates that the factor is released into the circulation almost immediately after the mating and reaches the critical level required for inducing ovulation in about 20 hours. Alternatively, when the female is normally mated (2 days after emergence) the critical level for ovulation is not reached until another week when the oocyte is mature.

Additionally, histological findings showed that the virgin females accumulate large amount of neurosecretory material (NSM) in the neurohemal organ, which indicates that mating is not necessary either for triggering synthetic activity or for transport of the NSM from the neurosecretory cells of the brain to the neurohemal organ.

The results suggested that the mating act may merely remove an inhibition to release the ovulation-stimulating hormone already present in the neurohemal organ, into the circulation, whereas the ovary with maturing follicle triggers synthesis of the hormone and/or transport thereof from the neurosecretory cells to the site of release.

Effects of juvenile Hormone and Juvenile Hormone Analogues on the pregnancy and embryogenesis in the tsetse fly, *Glossina morsitans morsians.*

M. F. B. Chaudhury and P. Osula

Under laboratory conditions (25±0.5°C; 60–80% R. H; 12L:12D) a female *G. m. morsitans* deposits a mature larva after each 9-day gestation period. The 9-day cycle includes about 4 days of embryonic development and another 5 days of *in utero* development of the larva. However, an egg or a larva may sometimes be expelled by the female before the normal gestation period has elapsed. Results of previous studies suggest that a neurosecretion from the pars intercerebralis of the brain may be responsible for such premature deposition of the progeny. In addition, certain nervous factors may also be involved in inducing such premature deposition of egg and larva (ICIPE Annual Report, 1978). As the underlying mechanism of abortion, we have suggested that the neurohormone responsible for inducing abortion may use cyclic AMP as a second messenger in triggering its response within the uterus (Denlinger *et al.* 1978). Previous work at ICIPE showed that the application of large dosages (10–50 μg/fly) of juvenile hormone analogues (JHA) will induce abortion in at least one cycle of 35% of the treated fly (Denlinger 1975). The objective of the present study was to determine the incidence of abortion in the pregnant females treated at various stages of the pregnancy cycle with juvenile hormone (JH) and various JHAs.

The hormone (JH III) and the analogues used (ZR 512, ZR 515 and ZR 777) were applied topically with 2 ul of acetone. Each pregnant female was treated with the hormone or one of the analogues (5.0 μg/fly) either once, twice or three times during the second pregnancy cycle. While the first treatment was applied 1 day after the first larviposition/ovulation, the second and third treatments were carried out on fourth (immediately after hatching) and eighth (with 3rd instar larva) day following the first larviposition respectively.

Results of the investigation showed that maximum reduction in progeny due to abortion (70%) was achieved when ZR 515 was applied once in the beginning of the pregnancy cycle and once again in the middle of the cycle. In comparison, JH III, ZR 512 and ZR 777 resulted in 62, 35 and 24% reduction respectively when applied in the same stages as in the case of ZR 515. Only one treatment, applied at the end of the pregnancy cycle, was least effective in inducing abortion (92% producing viable puparia); however, this treatment induced developmental arrest in the embryogenesis of the following egg in 15% of the treated females. An increase in dosage (10 μg/fly) resulted in arrest of embryogenesis in about 50% of the treated females. Abnormal eggs were expelled by the females.

From the results of this investigation it is evident that there is a definite sensitive period at which abortion can be induced by the treatment of ZR 515; the embryonic stage at which development can be arrested is dependent on the time of application.

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Control of Reproduction in the Male Tsetse Fly *Glossina morsitans morsitans*

M. S. Ramasamy

Most neurosecretory materials act as hormones and are important in the life of insects. It is known that insects use neurosecretory and other hormones to control their growth and development and the aim of the present investigation is to elucidate endocrine interactions controlling the development and function of the reproductive system in male *Glossina morsitans morsitans.*

The internal reproductive organs of the adult male consists of a pair of coiled testis, a pair of vas deferentia and a pair of accessory glands. The vas deferentia and accessory glands open into a common unpaired ejaculatory duct which continues to the gonopore. The innervations to the reproductive organs originate in the thoracic ganglion. During copulation accessory gland secretions are transferred to the uterus of the female to form a spermatophore within which the sperm is deposited.

At the outset it has been necessary to obtain basic information on the overall reproductive behaviour of the fly. This has involved repeating some of the earlier

work on *G. m. morsitans* and other species of *Glossina* carried out at ICIPE and in other laboratories. Investigations can be grouped as follows:

(a) The development of the reproductive organs and their relationship to the neuro-endocrine system;
(b) The neurosecretory system of the adult male;
(c) Control of accessory gland function;
(d) Spermatophore formation;

Histological studies show the presence of the testis in the larva. At pupation the testis is uncoiled and oval shaped but begins to elongate and coil on day 3. Spermatogonia divide actively to produce spermatocytes which are present in groups until day 6–8 when meiosis occurs. In a 9-day-old pupa the testis shows a marked change. They are coiled and contain spermatids in cysts. The vas deferentia and ejaculatory duct are well formed and the external genitalia are distinguished. The accessory glands are seen at the same time. They are very small and difficult to observe in section. From here on the spermatids undergo transformation. The flagella begin to elongate and until about day 14 spermatids are seen in various stages of development. Mature sperms are seen on day 15. There is no marked change in the appearance of sperms until 27–28 days when emergence occurs. At emergence, the testis are loosely coiled and the sperm inside immotile. The coiling becomes tighter around day 2 of adult life. The adult testis distinctly lacks a succession of zones containing cells in different stages of development. The accessory glands are well developed at emergence and they do not increase appreciably in length during the life of the insect. Very little secretions are present in the glands at the time of emergence. The diameter of the glands increases on day 3 with the accumulation of secretions in the lumen. It seems therefore, that growth (and differentiation) of the accessory glands takes place in the pupa but their full capacity for synthesis is attained only after adult emergence.

The neurosecretory system of the adult male is similar to that of the female (second annual ICIPE report). The median neurosecretory cells in the pars intercerebralis are recognised by their reaction with aldehyde fuchsin—which stains predominantly proteinaceous secretions. It is assumed that these cell bodies contain both stainable and physiologically active material. The median neurosecretory cells stained with aldehyde fuchsin are termed A cells. These A cells do not stain with aldehyde fuchsin in pupae or newly emerged males. However, stainable neurosecretions appear during day 1 of adult life. The nuclear and cellular diameters of A cells do not change significantly between day 2 and day 6 when the fly is sexually mature (see below) indicating that the synthetic activity of these cells is not increased.

The corpus allatum is large and well developed in the pupa. No neurosecretions are detected in the corpus cardiacum—corpus allatum complex throughout the pupal instar. Neurosecretory granules are seen in the neurohaemal area (aorta) of the adult at the time they appear in the A cells. Adult males fixed soon after copulation (and successful insemination) showed no depletion of stainable material in the median neurosecretory cells or in the neurohaemal area. This indicates a balance between release, transport and synthesis of neurosecretions i.e. new material being synthesised to replace what is released so that no depletion is observed. Alternatively, if neurosecretions are released on mating, it is possible that the amount of hormone present is not indicated histologically by the visible amount of neurosecretion. No cells in the thoracic ganglion were stained with aldehyde fuchsin.

Adult male *G. m. morsitans* are unable to inseminate until day 4. Newly emerged males will not copulate with receptive females. On day 3 (i.e. more than 72 hours after emergence) they copulate more readily and about 85% are able to inseminate within the next 24 hours. Very often 100% insemination is observed from then on, until 25 days. The accessory glands are seen to accumulate secretions and achieve their maximum diameter on day 3–4 when they contain white opaque secretions.

Accessory gland secretions and spermatophores collected from uteri of females soon after separation from copula were analysed by polyacrylamide gel electrophoresis. The protein composition of the accessory gland secretions appears essentially similar in sexually immature and mature males—the differences between them seem quantitative rather than qualitative. About 25 different proteins (molecular weights ranging from 15,000–200,000 daltons) have been detected in the secretions of adult glands. Many of these proteins were present in the spermatophore and no major new bands were observed. These studies are being continued.

The presence of accessory gland secretions and spermatophore formation are essential for sperm transfer. Males who had these glands surgically removed were unable to inseminate although they were in copula for over 2 hours. However, when only one gland was removed the secretions in the remaining gland were sufficient for spermatophore formation and sperm transfer. There is no appreciable depletion of material in the gland after mating and males were observed to successfully inseminate a second female soon after.

The role of the corpus allatum in the synthesis and transfer of accessory gland secretions was examined. It was reported in the fifth annual ICIPE report that allatectomy did not affect mating in adult males. This experiment was repeated. Allatectomised flies were examined for their mating potential. Such males showed no abnormalities in mating and could inseminate 10 females for over a period of 3 weeks. This suggests that the capacity to synthesise proteins and their later transfer are independant of the corpus allatum or a hormone secreted from it into the haemolymph. It is therefore,

not surprising that topical application of JH III had no effect in inducing sexual maturity or synthesis of accessory gland secretions. Similarly, fumigation with Precocenes I and II did not affect sexual maturation of young male tsetse flies. Experiments to determine the role of other neurosecretions and hormones in the synthesis of accessory gland secretions are in progress.

The innervation to the accessory glands was studied by staining them with methylene blue. Ultrastructurally, the axon endings on the accessory glands are seen to contain dense core granules and synaptic vescicles whose size suggest that they are aminergic. Preliminary pharmacological studies indicate the involvement of these amines in the release of accessory gland secretions. Further investigations are in progress.

Director of Research
Professor T. R. Odhiambo

Research Staff
Miss R. Chesang (1972) Junior Technician
Miss N. F. Darji (1974) Research Assistant
Dr. T. K. Golder (1978) Research Scientist
Dr. F. L. Lambrecht (1978) Principal Research Scientist
Mr. J. Likhanga (1974) Technical Assistant/Driver
Mr. E. Mpanga (1978) Technician
Dr. M. B. A. Nyindo (1976) Research Scientist
Mr. P. Onyango (1974) Technician
Dr. L. H. Otieno (1973) Senior Research Scientist
Mrs. N. Y. Patel (1975) Associate Scientific Officer

Introduction

The prevalence of *Trypanosoma* (*Trypanozoon*) *brucei* group organisms in Tsetse flies have been studied extensively and a lot of information is available on the importance of tsetse as vectors of pathogenic African trypanosomes. It is amazing, however, that there is very little known on the immunological, biochemical or physiological responses of tsetse to the ingested trypanosomes. Studies summarized below were undertaken to elucidate some of the initial barriers to *T. brucei* establishment in the tsetse midgut and subsequent development to mature salivary gland stages.

Infective development of *T. brucei* in Tsetse flies

L. H. Otieno, N. Darji and P. Onyango

(a) Age and temperature effect on *T.* (*T*) *brucei* infections in *G. m. morsitans*

A review of the available literature indicates that the percentages of mature infections with *T. brucei* subspecies obtained in tsetse flies in laboratory transmission experiments are invariably very low. Jenni (1977), was able to obtain high numbers of mature infections in *G. morsitans* by cooling newly emerged flies at 20°C for eight hours and subsequent maintenance at 25°C and 80% relative humidity. By modifying some of the procedures outlined by Jenni, we obtained up to 45% *T. brucei* mature infections in *G. morsitans*. Essentially, emerging *G. morsitans* were collected in PVC cages at 2 hourly intervals and fed on infected rats or rabbit at various time intervals (see Table 1). Soon after engorgement, the flies were cooled at 4°C for 30 minutes. After cooling the flies were returned to 25°C and 80% r.h. maintenance room. They were examined for trypanosome infection 28 days post infection. The results of these studies are summarized in Table 1. The table shows that flies fed on rats developed significantly higher (24.1%) mature infections than flies fed on rabbit (5.2%) ($G=12.54$, $P<0.001$, 2 df). Since the rats on which the flies fed had much higher parasitaemic levels than the rabbit, it would appear that the number of flies developing salivary gland infections is directly proportional to the number of trypanosomes ingested. Studies are in progress to see whether these differences may be due to host blood differences. The table also shows that flies fed when they were 7–8 hours old had the highest number of mature infections (45.4%). The table further shows that flies cooled after infective blood meal developed higher number of mature infections compared to control flies ($G = 3.81$, $P<0.05$, 1 df). However, neither cooling nor age influenced the infections in flies fed on rabbit.

(b) Crop emptying

Moloo and Kutuza (1970) showed that there was a direct temperature effect on the rate of tsetse crop emptying. They, however, used wild caught flies. We have made attempts to see if ambient temperature influences the rate at which young flies empty their crops, and by inference, the rate at which ingested trypanosomes would be migrating into the tsetse midgut.

Young flies of various ages were fed on clean rats then cooled for 30 minutes. They were then dissected at various time intervals and the state of the crop observed. Empty crops were recorded in those flies in which no more blood could be seen, and instead the blood had been replaced by an air bubble.

Figure 1 shows the results obtained.

The cooled flies tested 17–19 hours after emergence took twice as long to fully empty their crops compared to controls. No difference was seen in the rate of crop emptying for the first 2.5 hours, in test and control flies tested when they were 3–5 hours old. Thereafter the control flies emptied their crops much faster than the cooled flies. These observations showed that cooling flies influenced the rate at which they emptied their

Table 1. A comparative study of *T. brucei* transmission by *G. morsitans*. Flies of various age groups were fed either on rabbit or rat infected with *T. brucei* EATRO 1969. Soon after feeding group B flies were cooled at 0–4°C for 30 minutes and then transferred to room temperature (25°C). Group A flies were not cooled

GROUP A—CONTROL
GROUP B—TREATED

Rat Fed

Fed at hours after emergence	GROUP A No. examined	Proboscis	Gut	Salivary Gland	% Mature infections	GROUP B No. examined	Proboscis	Gut	Salivary Gland	%Mature infections
22–24	15	—	1	1	6.6	15	1	3	1	6.6
20–22	15	1	1	1	6.6	13	2	4	2	15.4
18–20	14	1	4	1	7.1	15	3	4	2	13.3
16–18	14	1	3	1	7.1	19	4	7	5	26.3
14–16	23	2	5	3	1.3	29	2	4	3	10.3
12–14	3	—	—	—	—	2	—	—	—	—
TOTAL	84	5	14	7	8.3	93	12	22	13	14.0
7–8	10	2	3	2	20.0	11	6	7	5	45.4
6–7	15	6	8	2	13.3	16	6	7	6	37.5
5–6	11	2	3	2	18.2	10	2	5	1	10.0
4–5	16	2	4	2	12.5	17	5	11	5	29.4
3–4	16	2	7	3	18.8	20	5	5	5	25.0
2–3	12	—	—	—	—	13	—	4	2	15.4
1–2	19	4	3	2	10.5	16	3	3	2	12.5
up to 1	12	5	8	3	25.0	13	2	7	2	15.4
TOTAL	111	23	36	16	14.4	116	29	49	28	24.1

RABBIT FED

Fed at hours after emergence	GROUP A No. examined	Proboscis	Gut	Salivary Gland	%Mature infections	GROUP B No. examined	Proboscis	Gut	Salivary Gland	% Mature infections
22–24										
20–22	18	—	—	—	—	17	—	1	1	5.9
18–20	17	—	2	—	—	15	—	1	—	—
16–18	17	—	—	—	—	15	—	2	—	—
14–16	18	—	2	—	—	16	—	3	—	—
12–14	5	1	1	1	20	2	—	—	—	—
TOTAL	75	1	5	1	1.33	65	—	7	1	1.54
7–8	6	—	—	—	—	7	—	1	—	—
6–7	6	—	—	—	—	9	3	3	1	11.1
5–6	8	1	1	—	—	10	—	1	—	—
4–5	5	1	1	1	20.0	5	—	—	—	—
3–4	8	—	1	—	—	4	—	1	—	—
2–3	6	—	1	—	—	8	—	1	—	—
1–2	6	2	2	2	33.3	10	2	3	1	10.0
up to 1	7	1	3	1	14.3	5	1	2	1	20.0
TOTAL	52	5	9	4	7.7	58	6	12	3	5.0

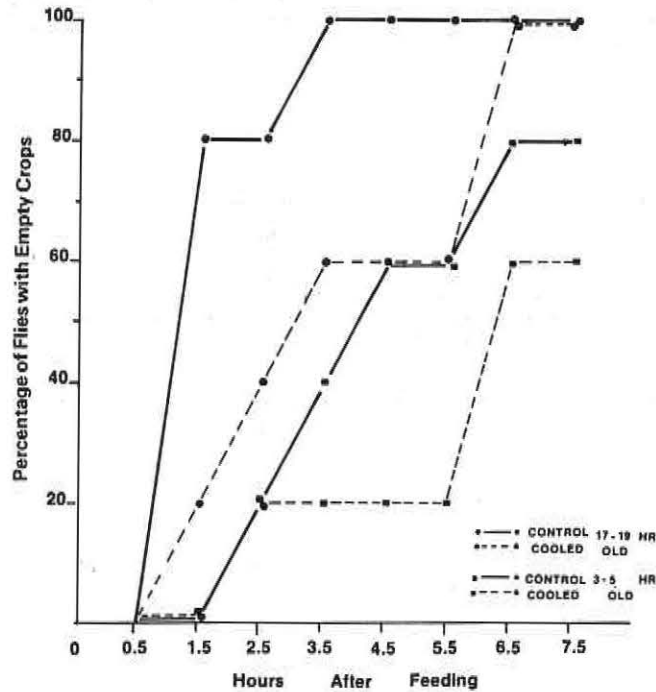


Figure 1. The effect of cold treatment on the rate of crop emptying in teneral flies. 3–5 and 17–19 hour-old flies are compared.

crops. It is reasonable to suppose that any trypanosomes ingested by the cooled flies would have a much longer time in the crop to adjust to the fly's internal environment before they are transferred to the midgut where they would immediately be faced with midgut proteolytic enzymes.

Studies are in progress to see whether trypanosomes undergo any physiological changes by prolonged residence in the crop.

Young newly emerged and three day old (fed twice before inoculation) *G. m. morsitans* were inoculated artificially with clean suspensions of *T. brucei*. Aliquots containing 10^7 trypanosomes/ml were then inoculated into the rectum by blowing in through the anus of the flies.

Table 2. Inducing *T. brucei* infection in *G. morsitans* by injecting trypanosomes through the anus. Susceptibility of young (8–12 hr old) and 3 day old flies to *T. brucei* infection both under natural and artificial situations are compared.

Flies Infected Artificially										Flies Infected Normally									
8–12 Hr-Old Flies Infections in					3 Day-old Flies Infections in					8–12 Hr-Old Flies Infections in					3 Day-Old Flies Infections in				
No. Exam.	Prob.	Gut	Haem.	S.G.	No. Exam.	Prob.	Gut	Haem.	S.G.	No. Exam.	Prob.	Gut	Haem.	S.G.	No. Exam.	Prob.	Gut	Ham.	S.G.
20	5	16	—	5	38	—	9	—	2	24	3	6	—	4	20	1	3	1	1
% Infected	25	80	—	25		—	23.7	—	5.3		12.5	25	—	16.7		5	15	5	5

(c) Artificially induced infections in *G. m. morsitans*

The inoculated flies were fed on a clean rabbit four hours after the treatment. Control flies were fed on the infected rat before it was killed for the inoculation experiments. The artificially infected and the control flies were subsequently maintained on one rabbit for 28 days after which they were killed and examined for the presence of trypanosomes. The results of this study are shown in Table 3. The table shows that three day old flies fed naturally (control) on infected rat and those infected by the anal route developed salivary gland infections. In both groups 5% mature infections were recorded. This was in sharp contrast to the results obtained with young flies, in which 25% mature infections were observed among artificially infected flies. Young flies fed naturally on infected rat developed 16.7% mature infections.

Table 3. Anal transmission of *T. brucei* by *G. morsitans*. The trypanosomes were introduced into the flies through rear (Anus) and the flies subsequently examined at various time intervals for the presence of live trypanosomes

Period (days) after inoculation	No. flies examined	Proboscis (L-labrum; H-phopharynx)	Gut	Haemolymph	Salivary Glands
1	6	5L	4	5	—
2	5	3L	3	4	—
3	5	1L	3	3	—
4	7	3L	3	4	—
6	7	2L	1	5	—
8	7	1L+H	2	2	—
10	7	1L	1	1	—
12	7	1L	2	2	—
14	11	—	1	—	—
30	51	5L+H	4	—	4
Total	113	22	24	26	4

The interactions between *Glossina morsitans morsitans* trypsin and aminopeptidase and midgut *Trypanosoma* (*Trypanozoon*) *brucei brucei*

R. M. W. Vundla, L. H. Otieno and E. Mpanga

The work aimed at examining the presence of interactions between the midgut forms of *Trypanosoma* (*Trypanozoon*) *brucei brucei* and the midgut proteases of *Glossina morsitans morsitans* has continued. The problem has been approached in several ways.

1. It has been shown that blood form *T. b. brucei*, when mixed with crude midgut contents of some *G. m. morsitans*, and incubated at room temperature, transform into forms morphologically resembling the vector forms of the parasite (Otieno, 1978). In this study, trypanosome suspensions were prepared from rat blood (Lanham and Godfrey, 1970). It was observed that on mixing such suspensions with midgut homogenates, the trypanosomes increased their motility tremendously, moving at velocities characteristic of midgut forms. Controls incubated in phosphate saline glucose (PSG) showed normal motility. Examination of stained preparations by light microscopy showed no change in the morphology of the trypanosomes. Furthermore, the trypanosomes were able to pass through a DEAE-52 cellulose column at pH 8.0, indicating that the surface coat was intact. Electron microscopy showed no changes in the surface coat, kinetoplast and mitochondrion.
2. The search for an inhibitor that would function *in vivo* for one or more of the midgut proteases, has continued. Such an inhibitor would be a useful tool in the study of the effect(s) of the proteases on the trypanosomes. 1–chloro-3–tosylamido–7–L–heptanone hydrochloride (TLCK), a trypsin inhibitor, was very effective *in vivo*. There was no apparent damage to the flies. TLCK also inhibited midgut haemolysin. This compound appeared to inhibit transformation of blood form trypanosomes to midgut forms, in newly emerged flies, membrane—fed on an infected meal containing TLCK at a final concentration of 0.02 mg/ml. The effect was directly proportional to the concentration of TLCK. However, further investigations showed that TLCK has a direct trypanocidal effect. Transformation was therefore probably arrested because the trypanosomes were not in their normal physiological state.
3. Cooling flies (0°) for 20 minutes immediately after being fed on an infected meal enhances the rate of infection tremendously (up to 50%) (Otieno, 1979). The activities of trypsin and aminopeptidase in flies cooled after feeding was compared with those of uncooled flies. There was no difference in the activities of trypsin and AP in the two groups of flies, indicating that the temperature effect is probably not mediated via the two enzymes.
4. An attempt was made to compare the activities of AP and trypsin in single flies in which salivary glands infection was established and those in which there was no infection. However, individual variations were very large, making the results difficult to interpret.

Localization and Characterization of Cholinesterase from the salivary gland of *Glossina morsitans*

T. Golder and N. Patel

During the last few decades there has been considerable interest and research into the properties of insect cholinesterases. This interest is mainly due to the widespread use of anticholinesterases as pesticides. The underlying theme of this type of research is that the enzyme may have some unusual property that can be exploited for specific insect control. There is an enormous amount of information available on the cholinesterases of insect nervous systems but little is known about the non-neural enzyme. We have

found cholinesterase (ChE) activity in the salivary gland of *Glossina morsitans* and have histochemically localized it and have examined some of its properties.

Histochemical localization of the ChE was achieved using a standard copper-thiocholine technique. Formalin fixed glands were incubated in a buffered copper sulfate solution which contained either acetyl-thiocholine iodide (ATC) or butyryl-thiocholine iodide (BTC) as substrate. The localization of enzyme activity involves enzymatic hydrolysis of the substrate which forms a thiocholine-copper precipitate. This precipitate is stabilized and rendered dark brown by subsequent treatment with potassium ferricyanide. Temporary whole mount preparations were observed mounted in glycerol. Prior to the addition of substrate, glands were incubated in the presence of one of several inhibitors. An inhibitor was also included in the reaction medium.

Polyacrylamide disk gel electrophoresis was performed on salivary gland homogenates to determine the number of molecular forms. The copperthiocholine technique of Karnovfsky and Roots was used to stain the gels for ChE activity. Also, a general stain for esterases was used which utilizes β-napthylacetate as substrate.

Salivary glands incubated in the presence of ATC or BTC show the same location of reaction product. Activity is localized between the epithelial cells of the secretory and absorptive regions of the gland. Each epithelial cell is surrounded at its lateral margin by ChE activity. The deposition of reaction product gives the stained gland a net-like appearance (Figs. 2 and 3). No reaction product has been observed in the duct region.

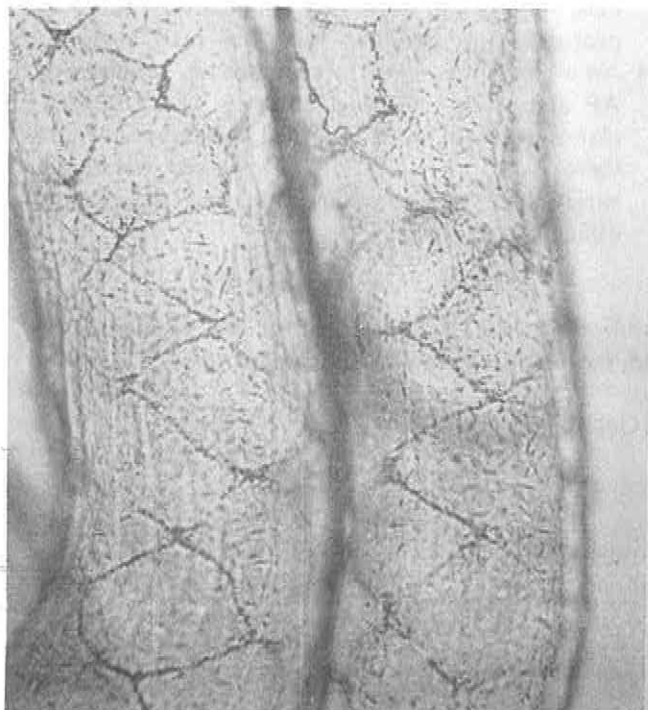


Figure 2. Whole mount of salivary gland of *G. morsitans* stained for ChE activity. The reaction product surrounds each epithelial cell giving the gland a net-like appearance.

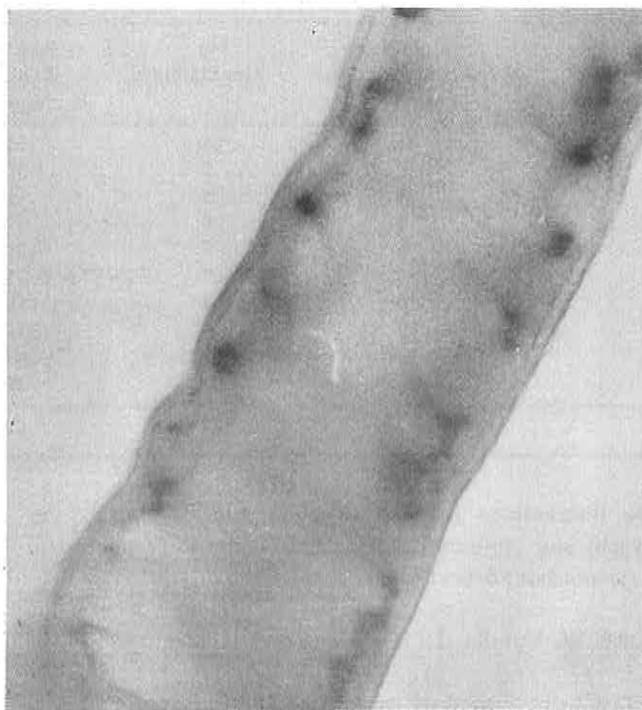


Figure 3. Whole mount of a salivary gland in optical longitudinal section. Note the position of the reaction product, just beneath the thin, external layer of muscle.

Glands from both male and female flies have been examined at ages ranging from newly emerged to fifty days old. The pattern of the activity is the same irrespective of the age or sex of the flies. In addition, recently fed or fasted flies also show the same ChE pattern.

To determine the type of ChE present, glands were histochemically stained in the presence of several inhibitors at several concentrations (Table 4). Tetraisopropylpyrophosphoramide (iso-OMPA) is a "selective" inhibitor of nonspecific or butyryl-cholinesterase (BuChE). It will inhibit acetylcholinesterase (AChE) to a minor extent. Note that iso-OMPA has a slight inhibitory effect on the enzymes ability to hydrolyze ATC at 10^{-4}M whereas the same effect on the hydrolysis of BTC is achieved at a 100 fold dilution (10^{-6}M). The "selective" inhibitor of AChE, BW284C51 [1,5—bis—(4 allyldimethylammoniumphenyl) pentan-3-one dibromide] shows the same inhibitory characteistics for both substrates. Eserine sulfate (physostigmine sulfate) is a very potent inhibitor of both AChE and BuChE at low concentrations (10^{-5} or lower) but shows no effect on arylesterases or carboxyesterases (aliesterases). Note that some inhibitory effect is evident at 10^{-8} and 10^{-9}M

Salivary gland homogenates run on 7.5% polyacrylamide gels show a single molecular form with low electrophoretic mobility (Fig. 4). The non-specific esterase stain as well as the ChE stain show the same and only band.

Table 4. Histochemical results for ChE activity of salivary glands of *G. morsitans* incubated in the presence of several inhibitors at several con centrations ++=very dark stain +=dark stain −+=light stain −=no stain

Inhibitor	Substrate	Concentration of inhibitor (M)					
		10^{-4}	10^{-5}	10^{-6}	5×10^{-6}	7×10^{-6}	
ISO-OMPA	ATC	+	++	++	++	++	
	BTC	—	—	+	+	+	
		2.5×10^{-5}	5×10^{-5}	10^{-6}	5×10^{-6}	10^{-7}	10^{-8}
BW284C51	ATC	—	—	—	+	++	++
	BTC	—	—	—	+	++	++
		10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	
Eserine	ATC	—	—	—	−+	−+	
Sulfate	BTC	—	—	—	−+	−+	

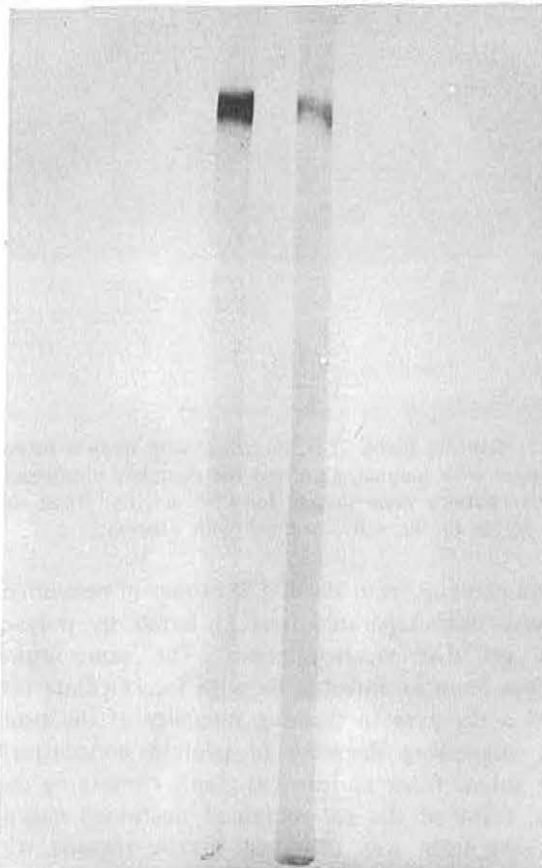


Figure 4. Polyacrylamide gels stained for ChE activity. The gel on the left is stained in a general esterase method using β-napthylacetate as substrate. The gel on the right is stained by the copper-thiocholine technique of Karnovsky and Roots. The origin is at the top.

These results indicate that there is a single enzyme present with properties most similar to AChE. The extreme sensitivity to eserine sulfate rules out the possibility that the reaction product is due to arylesterase or carboxyesterase. That iso-OMPA at fairly high concentration (10^{-4}M) has only a minor effect or hydrolysis of ATC yet the same effect on the hydrolyses of BTC can be achieved at a 100 fold dilution, suggests that the enzyme has a much greater affinity for ATC. These results also indicate that the ChE pesent is typical of insect cholinestrase in its ability to hydrolyze several substrates.

Some effects of trypanosome development on the salivary glands of *Glossina morsitans*

N. Patel and T. Golder

It has been reported (ICIPE Annual report, 1978) that the chemical composition of the salivary secretion of *Glossina morsitans* changes when the glands contain metacyclic trypanosomes. However, little is known about what secretory substances are utilized during trypanosome development and what effect trypanosome development has on the normal physiology of the gland. It has been observed that heavily infected glands lose their motility and flies thereby infected lose their ability to salivate normally. The observed changes in saliva chemistry and gland behaviour have prompted us to investigate the relationship between these changes and the maturation process of the trypanosomes.

Flies with salivary gland infections of *T. T. brucei* were obtained by using Otieno's (1979) cooling method. About 15–20% infected flies were obtained. The flies were persuaded to salivate every other day on clean microscope slides, and the saliva examined for parasites after giemsa staining. The flies showing parasites in the saliva droplets were grouped according to the presence of either immature forms of parasites, intermediate forms or mature forms. Selected glands were stained for cholinesterase (ChE) activity as reported above (Golder and Patel) at various time intervals after the first sign of parasites in the saliva. Saliva was collected using the bat wing technique and the protein pattern studied by polyacrylamide disc electrophoresis. Infected salivary glands having immature forms (Fig. 5) of parasites showed normal localisation of ChE activity (Fig. 5). In the salivary glands having intermediate forms of trypanosomes, the normal pattern of ChE activity was broken and incomplete in the glandular region. Slight activity was also found in the lumen and the duct region (Fig. 6). Glands with mature parasites showed high ChE activity in the lumen and the normal net like pattern disappeared completely from the epithelium (Fig. 7). ChE activity was also seen in the lumen of the duct region of the gland.

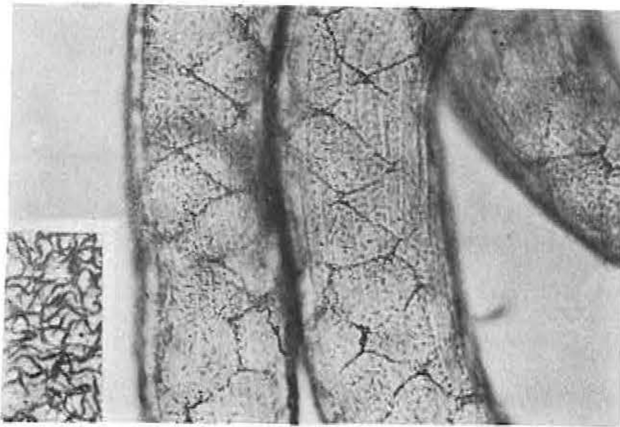


Figure 5. Salivary gland of *G. morsitans* with immature forms of *T. T. brucei* with normal pattern and without luminal stain when stained for ChE activity. Inset shows immature forms in the saliva stained with Giemsa.

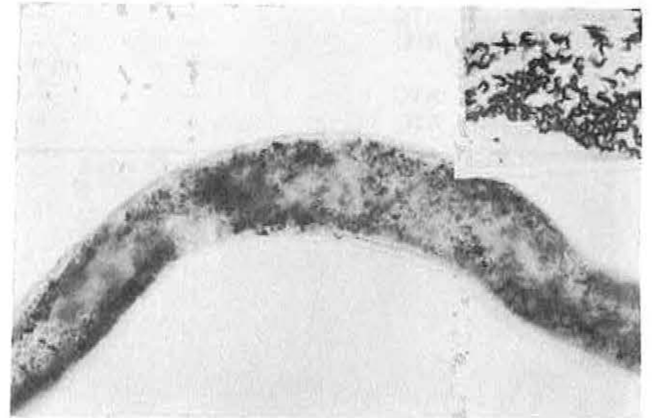


Figure 7. Salivary gland of *G. morsitans* with mature forms of *T. T. brucei* with luminal stain and the complete disappearance of net-like pattern when stained for ChE activity. Inset shows mature forms in the saliva stained with Giemsa.

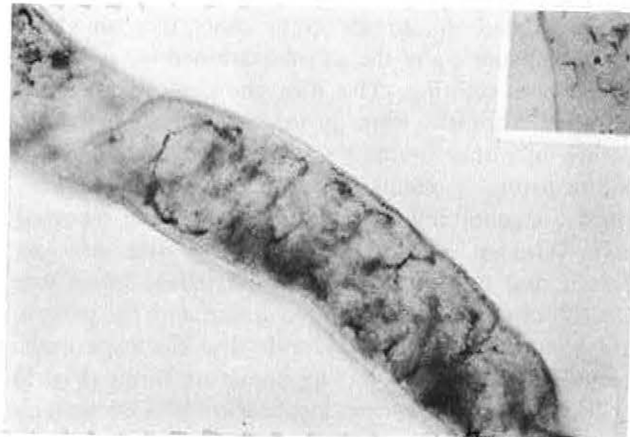


Figure 6. Salivary gland of *G. morsitans* with intermediate forms of *T. T. brucei* with broken pattern when stained for ChE activity. Inset shows intermediate forms in the saliva stained with Giemsa.

Saliva proteins from about 120 probes of non-infected flies have been separated into 11 bands by polyacrylamide gel disc electrophoresis. The same number of probes from an infected fly with intermediate forms showed a decrease in staining intensity of the protein bands, suggesting decrease in protein concentration. In the saliva from an infected gland containing metacyclics, most of the gel remained unstained except a very faint stain was observed in the regions which normally show the most intensely stained bands (Fig. 8). All the protein bands showed when about 720 probes of saliva containing metacyclics were used. In addition an extra band was seen below the first band.

The results strongly indicate that changes in protein composition of saliva and distribution of ChE of the glandular tissue correlate, with maturation of the parasite. It appears as though there is no selective dimunition of proteins during this transformation, but a general decrease in protein concentration.

It is not known whether this dimunition of proteins is the result of utilisation by the developing trypanosomes or merely a decreased output by the salivary gland. Similarly it is also not known whether the extra band of protein that appears in infected saliva is a product of the trypanosomes or represents the ChE lost from the gland. However, these results plus observations that glands with heavy, mature infections lose their

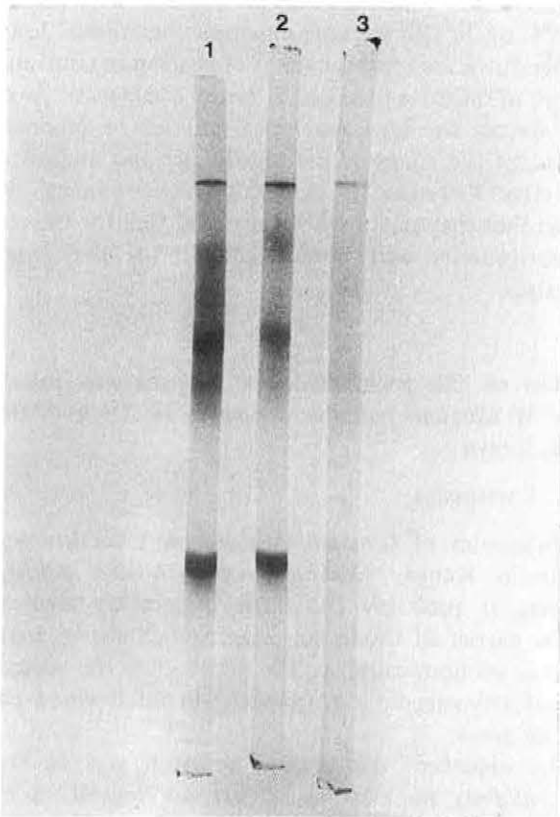


Figure 8. Major protein bands from 120 probers of saliva from (1) a non-infected fly (2) an infected fly with intermediate forms of parasites and (3) an infected fly with mature forms of parasites.

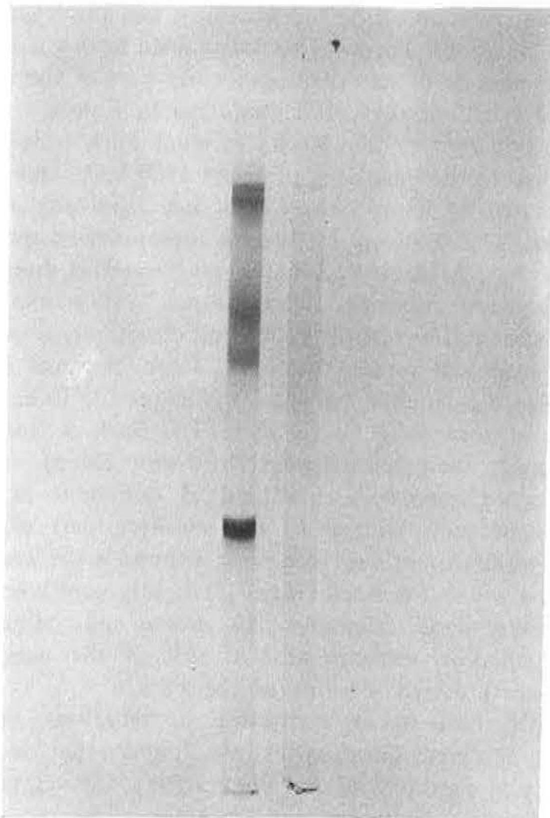


Figure 9. Major protein bands from an infected fly with mature forms (1) 720 probes of saliva (2) 120 probes of saliva.

motility suggest that the normal function of the glands are reduced and that a stress condition exists. Investigations are in progress to test this idea and find ways to exploit this condition for control of trypanosomiasis.

Cultivation *in vitro* of metacyclic trypanosomes in continuous culture

M. Nyindo and R. Chesang

Introduction

In the past 2 years of research it was reported that infective forms of *Trypanosoma brucei* were cultivated from the midgut and salivary glands of *Glossina morsitans morsitans* at 28°C and 38°C respectively. Recently, considerable progress has been made, in the *in vitro* propagation of metacyclic or infective forms of *T. brucei* from salivary gland explants of infected tsetse flies at 25°C and 30°C.

Experimental Protocol and Results

Rats were infected with *T. brucei* EATRO 1969, and at the first peak of the parasitemia newly emerged *G. morsitans morsitans* were fed on the animals. Development of parasites in the salivary glands was detected by examination of the saliva from the flies and by the ability of the tsetse flies to transmit the parasite to susceptible mice. On days 46 and 60 positive flies were chilled and their salivary glands were removed and placed into sterile plastic tissue culture dishes (T-flasks) containing bovine embryonic spleen cells in RPMI 1640 medium supplemented with 20% foetal bovine serum, 5% lactalbumin hydrolysate and antibiotics in standard concentrations. Cultures were incubated at 25°C or 30°C and examined daily for the development of parasites in the culture medium and salivary glands. Usually it took 3 to 4 weeks for parasites to increase in number at which time it became necessary to subculture the parasites. Subculturing was then done 3 times a week. Two types of parasites grew from this tissue culture system: One type was long and slender (Fig. 10), another type was short and stout.

Both types doubled their population in 8 to 10 hours at 30°C. Both types had a free flagellum and the kinetoplast was subterminal. Infectivity studies (Table 5) were carried out in rats and mice, and on one occasion in rabbits and cattle.

The parasites have been infective to rats and mice up to day 435 of testing and they also infected rabbits and cattle.

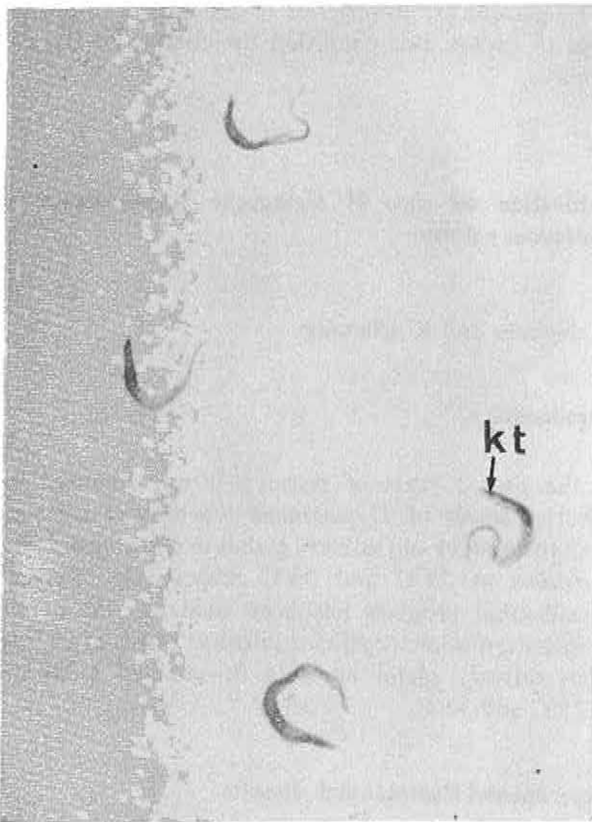


Figure 10. The long and slender parasites on day 98 of cultivation. The subterminal kinetoplast (Kt) is shown. X 1,850.

Table 5. Infectivity titration in rats of the long and slender metacyclic trypanosomes of *Trypanosoma brucei* cultured *in vitro* at 30°C.

Quantity of Parasites injected	Infection rates against number of animals injected	Percent mortality rate	Mean prepatent period in days
10^6	6/6	100	5
10^5	6/6	100	7
10^4	6/6	100	5
10^3	6/6	100	9
10^2	5/6	83	12
10^1	4/6	66	12

Six rats were injected intraperitoneally with parasites on day 238 of cultivation. Parasites were not passed through DE 52 before inoculation. Presence of parasites in peripheral blood was detected in tail blood for 30 days after inoculation.

Summary and significance of the study

Initial cultivation attempts of infective forms of *T. brucei* from the salivary glands of tsetse flies were carried out at 38°C. The resultant parasites did not maintain their infectivity to rodents for a long period in the course of cultivation. When subsequent cultures were initiated at 25°C or 30°C it became apparent that these "lower" temperatures are ideal for the propagation in continuous culture of infective forms of *T. brucei* (metacyclic forms). The system we have developed provides a laboratory model for the study of the physiology and anatomy of inefective *T. brucei* in an artificial environment. It is hoped that the system will be a useful tool for the study of antigenicity and immunogenicity of the Nagana parasites.

Studies on the populations and trypanosome infection rates of *Glossina pallidipes*, Austen, in Meru National Park, Kenya

F. L. Lambrecht

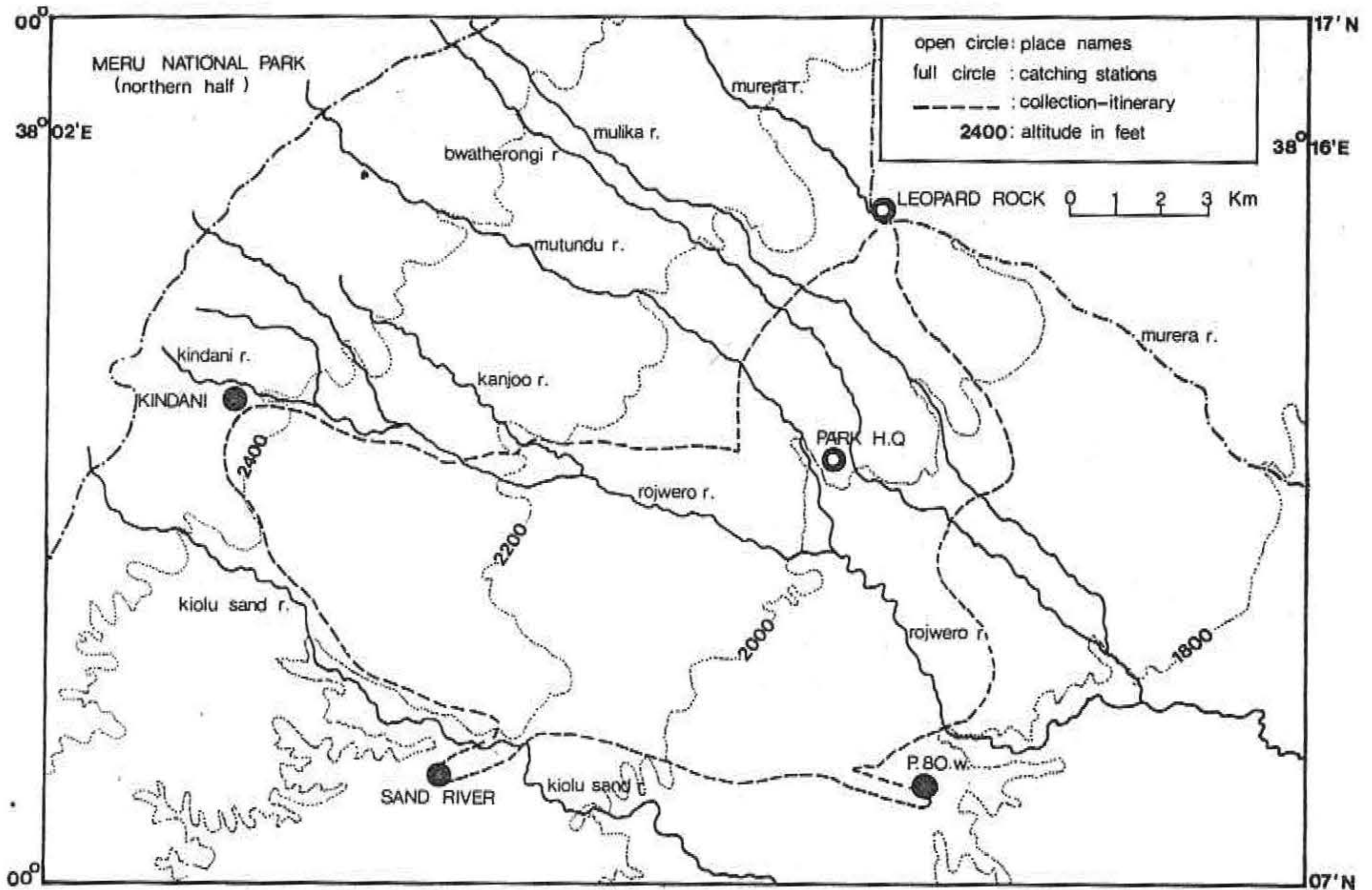
Seven species of *Glossina* (Wiedemann) are known to occur in Kenya. Among them, *Glossina pallidipes*, Austen, is probably the most important; medically, as the carrier of rhodesian sleeping sickness in western Kenya, economically, as the most effective vector of animal trypanosomiasis (nagana) in all lowland cattle raising areas.

The object of the present research was to study. *G. pallidipes* populations in various vegetation communities in the natural floral and faunal environment of Meru National Park over a period covering all seasons of the year. This with a view to obtain information on the environmental factors that would influence population composition and dynamics, and also trypanosome infections. The baseline data gained from a natural flybelt outside human disturbance are part of the integrated ICIPE studies of *G. pallidipes* in Kenya.

G. pallidipes in the Meru National Park is largely confined to the Park area of about 1500 km². This belt is isolated by many hundreds of km. from any other fly belt. The relatively small park contains most species of the East African Wildlife and an interesting diversity of woodland savannah, and soil types. Well-maintained road system traverses the Park in all directions, allowing easy access to various biotopes. Meru National Park stradles the equator between 0°20'N and 0°10'S, and extends from 38°00 to 38°25'E. The Park is roughly triangular, the base facing the Northwest (Map).

Acacia-Commiphora bushland is dominant in the basement rock section in the southern part of the park while Combretum woodland is found in the western section on well-drained ridges of slightly acid volcanic soil. Woodland dominated by *Acacia* spp. coincides with alkaline volcanic alluvial soil of the northern and northeastern sections of the Park.

With these major vegetation communities, other, more confined floral types are found: the riverine gallery along the Sand and Tana Rivers, the vegetation on the rocky inselbergs, and the patch of ground-water forest along the upper part of the Kindani River near the western border of the park.



Following preliminary surveys in Meru National Park during late 1978, routine collections and dissections of *Glossina* were carried out in the months of February, March, May, June, August, October and December, 1979.

The number of flies dissected during these periods was between 700–800 a week. Usually a higher number of flies were caught, some being preserved for later morphological studies. The last afternoon catch was taken alive to the ICIPE Nairobi Centre for various experimental work that included studies on sound production, and the isolation of trypanosomes from infected flies. Three trapping sites were selected and flies collected by means of the biconic trap. The number of traps at each site varied, the principal aim being to collect representative samples from each site.

Catching sites were established in three ecologically different areas:

1. Kindani, a tall, dense ground-water forest at an altitude of about 850m. (2,450 ft.). with an annual rainfall (1978) of 1,290mm., coordinates: 0°12'N–38°05'E.
2. Kiolu (Sand) River, a forest gallery at the limits of the *Acacia-Commiphora* woodland. Altitude about 700m. (2,100 ft.), annual rainfall (1978) 980mm., coordinates: 0°07'N–38°07'E.
3. Post 80 (waterhole), within the *Acacia-Commiphora* woodland. The waterhole started drying up in June and was completely dry by August. It started filling up again during the rains in November. Altitude about 600m. (1,800 ft.), annual rainfall (1978) 890mm., coordinates: 0°07'N–38°12'E.

Tsetse ready for dissections were kept in the freezing compartment of a paraffin-operated small frigidaire for about 10–15 minutes. This was to make them inactive so that legs and wings could be removed easily. The pair of wings was placed in a drop of saline on a slide and examined under the dissecting microscope for age-estimation by the wing-fray method. The fly was lightly pressed by means of forcepts and the haemolymph thereby extracted from the body cavity, deposited on a slide and numbered with a diamond pen—5 to 6 smears to a slide. The preparations were then fixed with methanol, later stained with Giemsa and examined for the presence of trypanosomes. Preparations from infected flies found in the course of the other examinations were also kept, fixed with methanol and later stained with Giemsa for confirmation of *in vivo* examinations. The gut contents of flies with recognizable red blood cells were collected on filter paper, numbered and preserved for later serological examination and identification of the bloodmeal.

Flies were dissected and examined by a team of three, one dissecting and examining the ovaries of female flies for estimation of physiological age; the second dissecting the mouthparts, salivary glands and

gut; the third examining these preparations for trypanosome infections. An average of fifteen flies per hour could be processed.

The studies are scheduled to continue at least until February 1980, it is therefore too early to present a full report.

Some of the data so far gathered are represented graphically in Figures 11, 12, 13 and 14; (11) total infection rates; (12) sex ratio; (13) relative "trapping density"; (14) age groups.

Infection rates compiled over the six surveys show the following figures: Kindani, 7.8%; Sand River, 2.4%; P.80.w., 5.3%. Average infections from all study areas show about 35% of the infections occur in males and about 65% in females. At present, this is distributed as follows: Kindani: 2.7% in males, 4.6% in females; Sand River: 1.80% in males, 3.88% in females; P.80.w.; 3.74% in males, 6.83% in females.

These infections occurred in the following proportion: in males: 55.8% in proboscis, 18.6% in gut, 25.5% in both gut + proboscis; in females: 50.0% in proboscis, 11.2% in gut, 38.7% in both gut + proboscis. No salivary gland infections were found in the 2736 flies dissected during this study. The total infections in the dissected flies averaged 4.5%. In most cases more females than males were caught in the biconic traps (figure 12). It was also observed that more flies were caught in the afternoon than during the morning. In October, the end of the dry season, flies decreased at Sand River and at P.80.w. This was not the case at Kindani where they increased markedly.

The map shows the northern part of Meru National Park in which the study areas were located. Figure 15 is a schematic representation of the relationship between topography, geology, vegetation and rainfall, and the sites of the trapping areas.

Table 6. Monthly rainfall at the three trapping sites in 1979(*)

Month	Kindani (2400 ft)	Sand River (2200 ft)	Waterhole (1800 ft)
January	136mm	136mm	153mm
February	12mm	5mm	6mm
March	76mm	65mm	57mm
April	274mm	228mm	189mm
May	83mm	40mm	16mm
June	1mm	0mm	1mm
July	0mm	0mm	0mm
August	0mm	0mm	0mm
September	0mm	0mm	0mm
October	4mm	2mm	1mm
November	30mm	27mm	22mm
December	13mm	7mm	4mm
January ('80)	0mm	3mm	0mm
February	0mm	0mm	0mm
Total	629mm	513mm	448mm

(*) Measurements from the nearest rain-gauge.

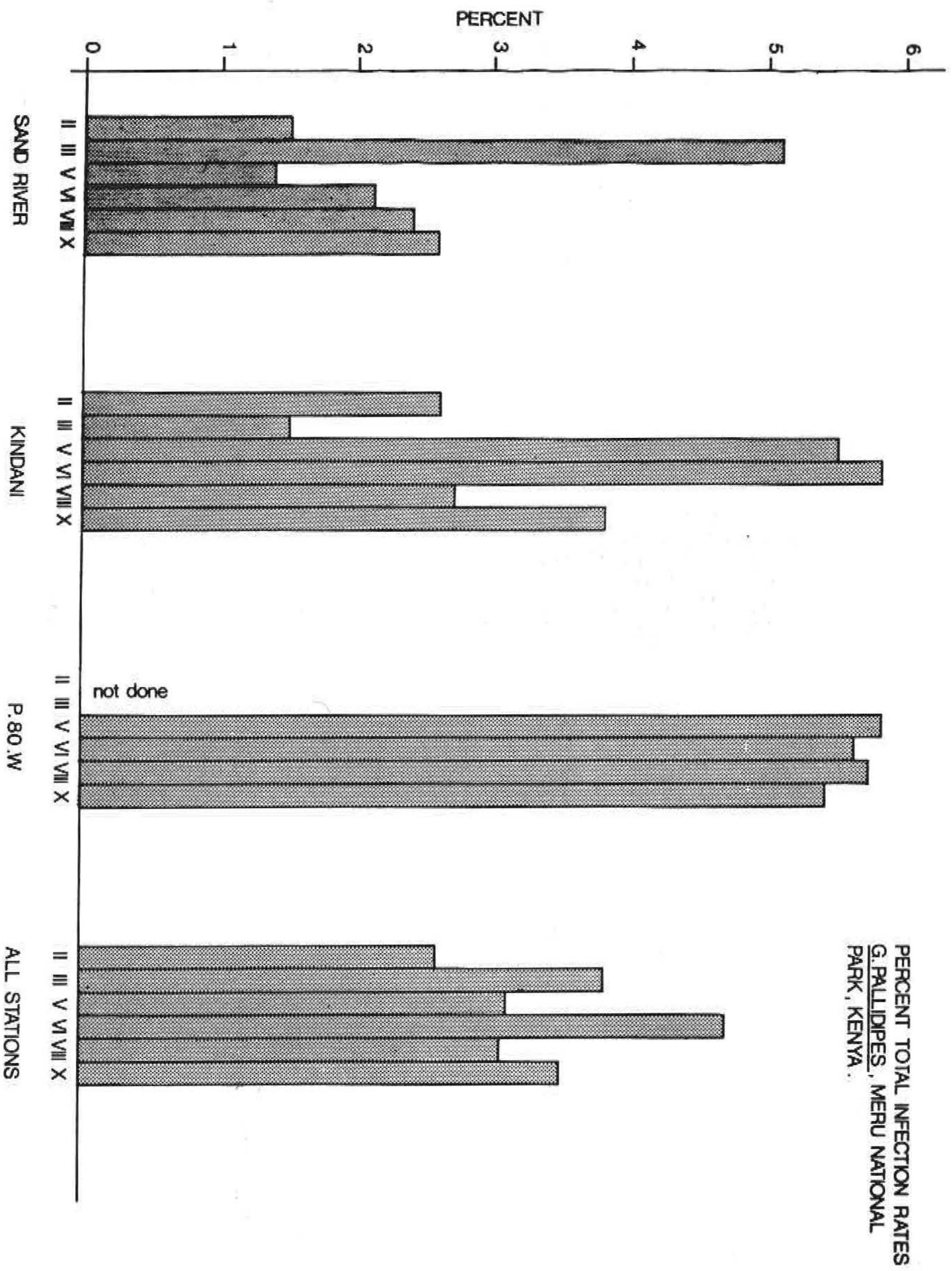


Figure 11. Monthly total infection rates: all trypanosome species, all parts of *G. pallidipes*, male and female flies.

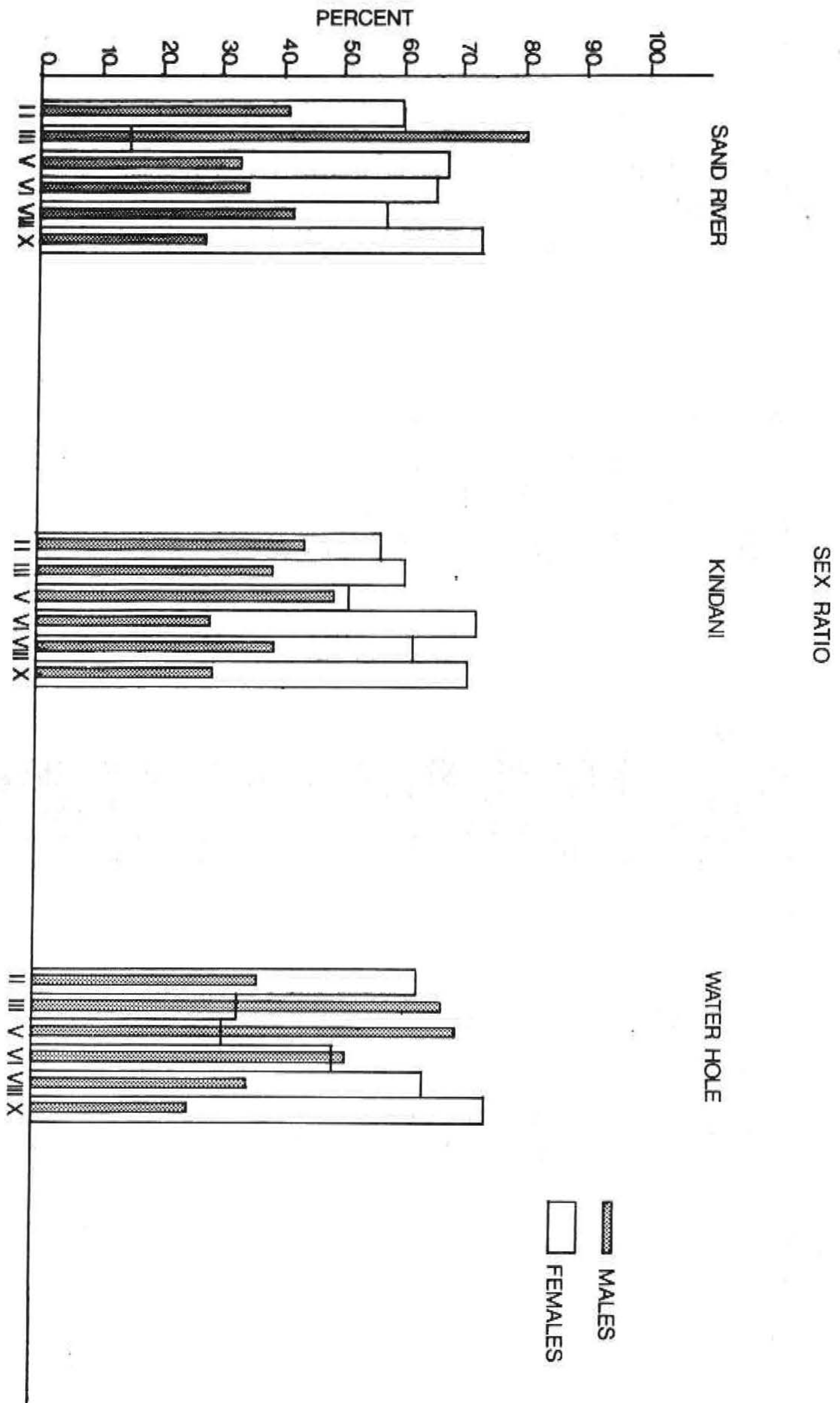


Figure 12. Monthiy Sex ratio of *G. pallidipes* caught in biconic traps, morning and afternoon catches combined.

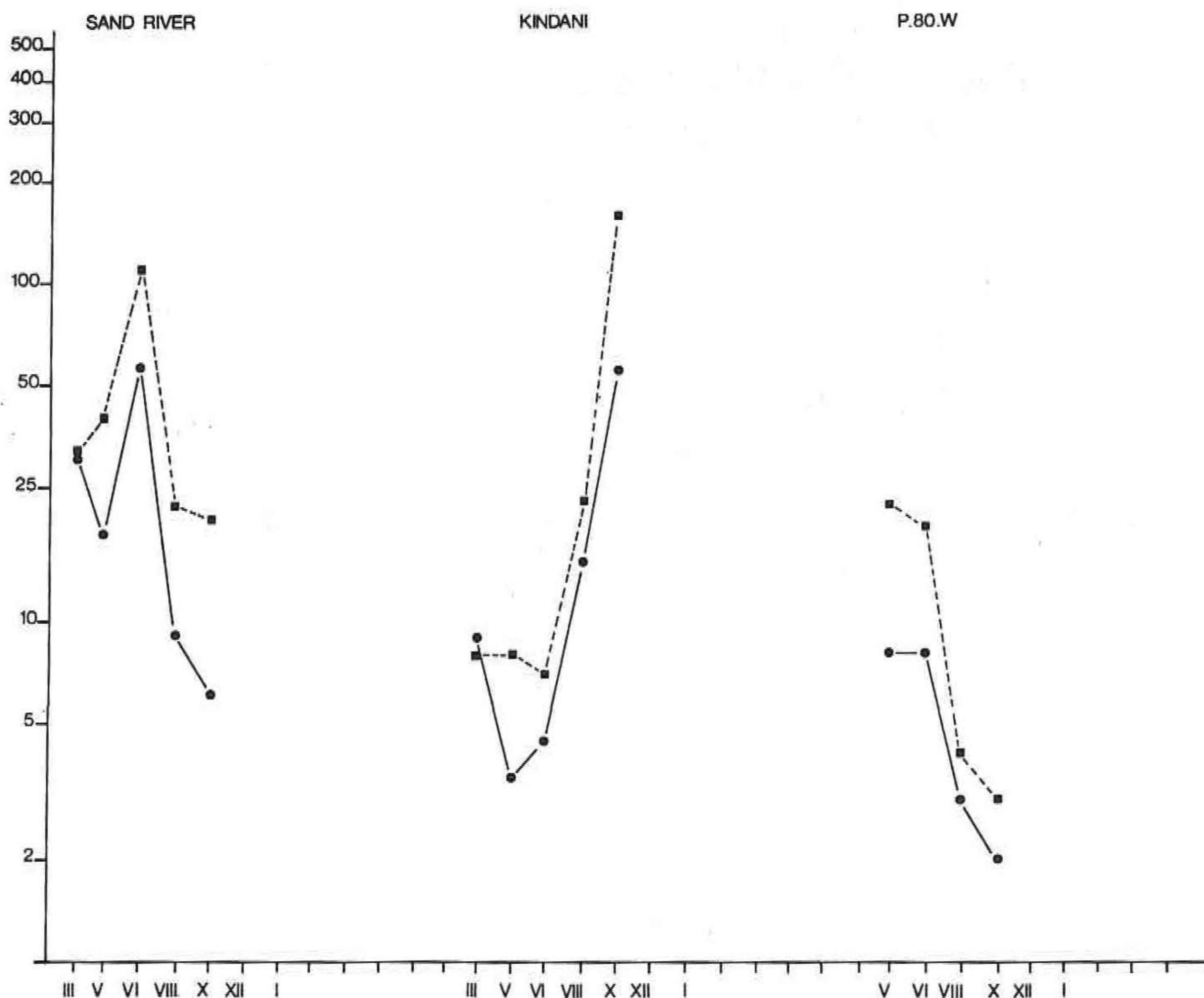


Figure 13. Monthly apparent density expressed by number of *G. pallidipes* flies caught in one biconic trap during a 24-hours period (● ——— males; ● - - - - females).

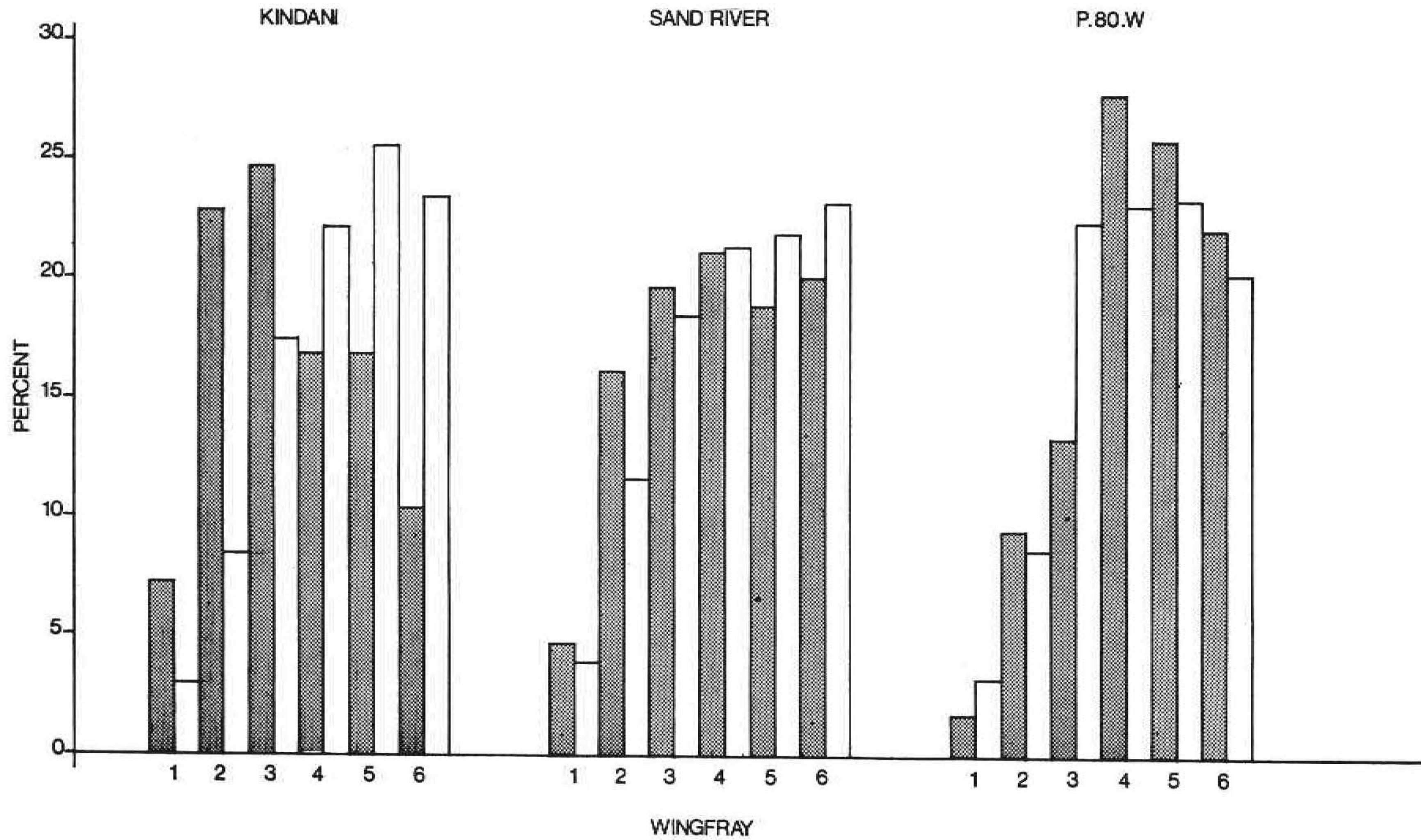


Figure 14. Age-category determined by wingfray (Jackson, 1946) for male and female *G. pallidipes* caught in biconic traps.

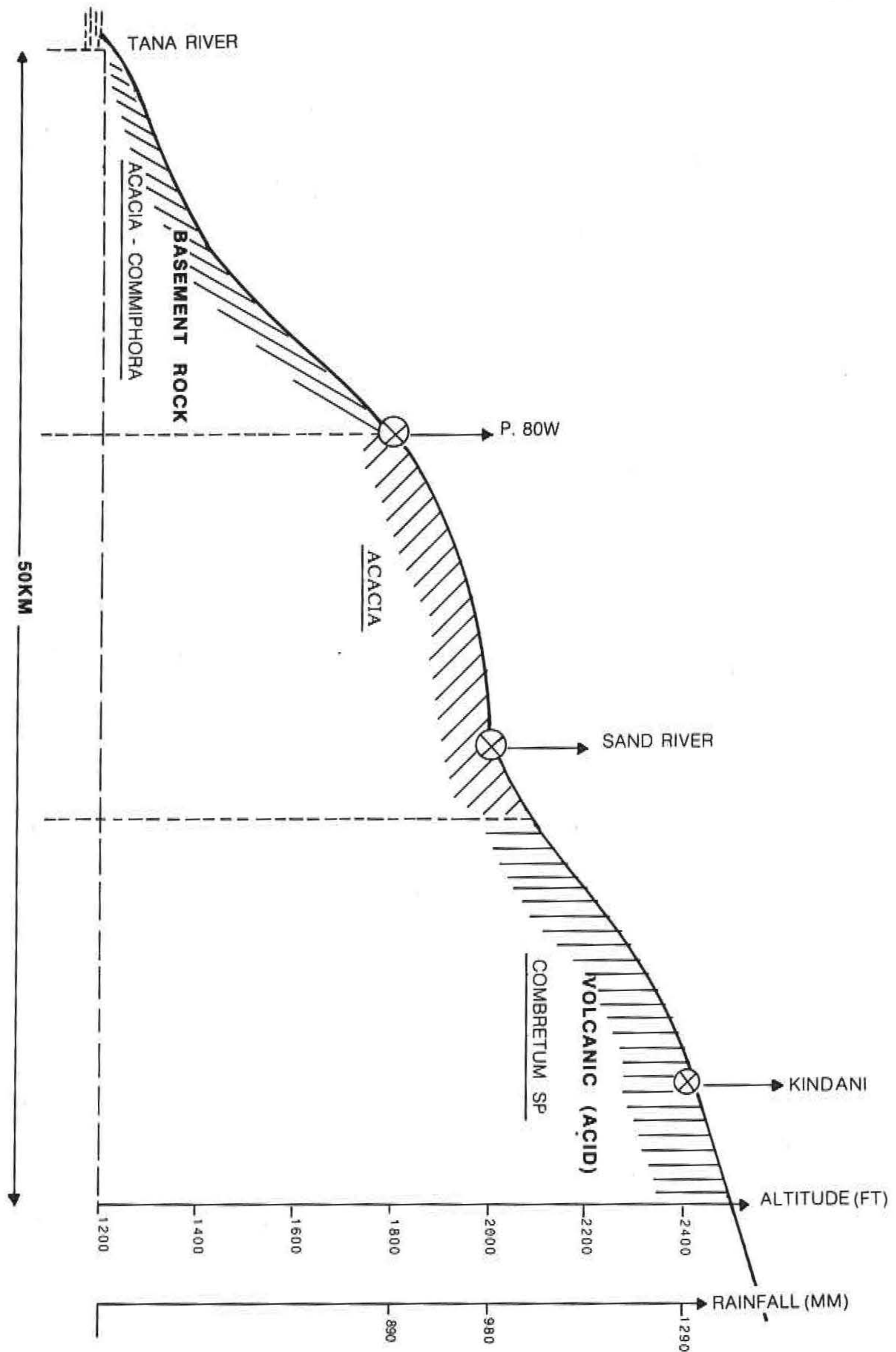


Figure 15. Schematic representation of soils, rainfall, altitude and vegetation in relation to the three study sites.

Research Staff

Mr. J. O. Apale (1974) Technician
Dr. J. van Etten (1974) Research Scientist
Mr. F. Kathuli (1977) Technical Assistant/Driver
Mr. J. K. Kiilu (1976) Subordinate Assistant
Mr. A. Makau (1976) Technician
Mrs. K. H. M. El. Malik (1979) Research Scholar
Mr. J. M. Muchiri (1979) Technical Assistant/Driver
Mr. D. K. Mungai (1978) Technical Assistant/Driver
Mr. R. Mutuaruhiu (1979) Junior Technician
Mrs. M. Owaga (1977) Scientific Officer
Dr. W. F. Snow (1977) Research Scientist
Dr. D. A. Turner (1978) Research Scientist
Mr. D. F. Uvyu (1974) Junior Technician

Population Diversity in the Tsetse fly

Glossina pallidipes Austen

Jaap van Etten

During the year laboratory studies on the spontaneous activity of *Glossina pallidipes* from the two study areas, Nkruman and Mwalewa, were completed. The study on genetic variation in populations of *G. pallidipes* has been extended to 8 populations, which form a good sample of the major populations of this species in Kenya (Annual Report, 1978). The performance of the Mwalewa colony over the past year has been briefly described.

Spontaneous activity

Initial results on the study of spontaneous activity, carried out at 24°C and 70% RH, with males of at least the second laboratory generation of flies originating from Nkruman and Mwalewa have been reported (Annual Report, 1978). These studies have now been completed, at 24°C, as well as at 30°C, and 70% RH in a LD rhythm of 12:12 and lights on at 6 a.m. local time. Males from both areas, which were used in the experiments, were of the same age, blood intake, and feeding frequency. Males were put in the actograph in the afternoon of the day they had fed, and recording started on the next day (referred to as day 1).

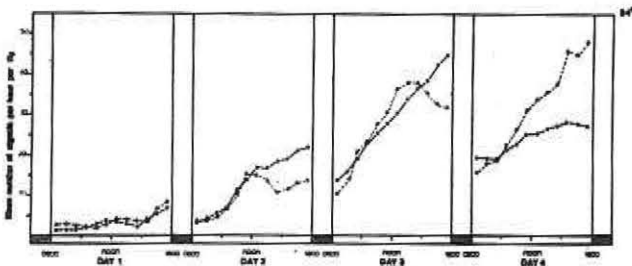
The total daily activity increased with increasing hunger, both at 24°C and at 30°C. An exception is the activity on day 4 at 24°C for males from Nkruman, which is lower than on day 3 (Table 1).

No significant differences were found in the total daily activity of males from the two areas during the first 3 days at 24°C, but on day 4, males from Nkruman had a lower activity. At 30°C. however, the total daily activity of males from Nkruman was significantly higher than of males from Mwalewa.

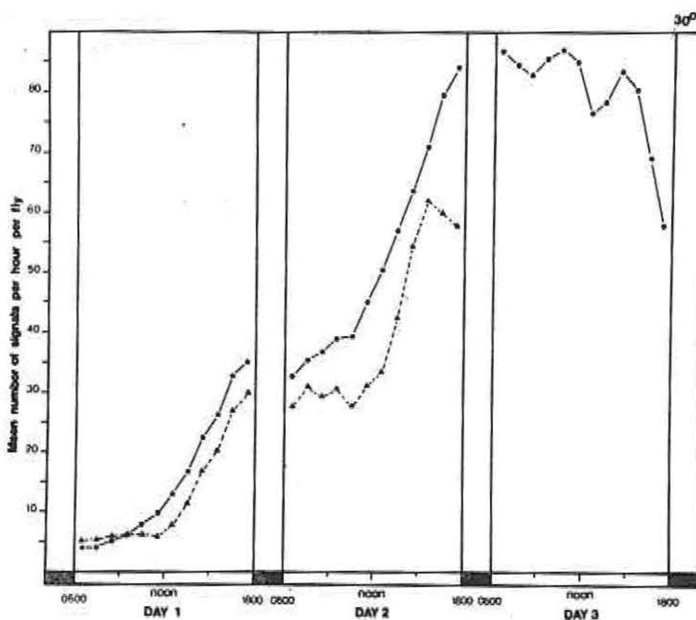
Table 1. Spontaneous activity of *G. pallidipes* males, originating from Nkruman and Mwalewa, expressed as average total daily number of recorded signals ±standard error at 24 and 30°C. n = number of experimental males

Number of days after feeding	24°C Nkruman		24°C Mwalewa		30°C Nkruman		30°C Mwalewa	
	n	Average number of signals ±SE	n	Average number of signals ±SE	n	Average number of signals ±SE	n	Average number of signals ±SE
1	21	39.2±9.0	20	48.7±7.2	20	185.9±29.2	20	151.4±35.6
2	22	160.4±37.0	21	126.2±28.4	16	641.3±90.6	8	504.8±134.5
3	19	351.1±79.5	17	344.4±88.0	4	970.3±153.8	1	(1120)
4	10	292.8±42.4	12	382.0±75.3				

The patterns of activity at both temperatures are shown in Fig. 1. Males from Nkruman had an increase in activity throughout the day for all days for both temperatures. The rate of increase in activity rises with increasing hunger, with exception of day 4 at 24°C. At 30°C the Mwalewa males had an activity which was more or less constant till noon, after which the activity increased sharply till the end of the light period on day 1, and till 4 p.m. on day 2, after which the activity declined.



1a



1b

Figures 1a and 1b. Spontaneous activity of males from Nkruman (solid line) and Mwalewa (broken line) during 4 days after feeding under constant laboratory conditions of 24°C and 70% RH (Fig. 1a) and 30°C and 70% RH (Fig. 1b).

Only a few males survived day 3 at 30°C, one male from Mwalewa, and four males from Nkruman. The Nkruman males had a high activity at the onset of the light, which gradually declined throughout the day, with a sharp decline after 3 p.m.

At 24°C, males from both areas had the same survival rate. However, at 30°C, males from Nkruman survived significantly ($P < 0.05$) longer than males from Mwalewa. This might suggest that the optimal conditions for flies from Nkruman are at higher temperatures than for flies from Mwalewa. This is also supported by field observations.

The laboratory results suggest that the activity patterns are influenced by temperature and hunger. The results also make it possible to understand the difference in diurnal pattern as observed in the field. The increase in activity under laboratory conditions in flies from Nkruman, occurred independently of temperature and hunger. A late afternoon peak is also observed under field conditions, although the pattern as a whole was different from the patterns in the laboratory. The field pattern showed an increase till around 10 a.m. followed by a slight decrease. From noon the activity increased up till dark. This reduction in midday activity is not observed under laboratory conditions, and might be caused by high temperatures (above 30°C,) or higher intensity.

Under laboratory conditions flies from Mwalewa showed a high afternoon activity only when they were very hungry, or at high temperatures (30°C). However, under field conditions flies from Mwalewa seem to avoid high temperatures, and will normally not reach the very hungry stage, as the high fat reserves of field flies suggest (ICIPE Annual Report, 1977). It might then be expected that at low temperatures in the field, flies from Mwalewa would have a peak activity around midday as was indeed observed. Under field conditions, flies are able to avoid activity at high temperatures, by looking for resting sites, which provide a microclimate with lower temperatures. This means that an increase in activity after midday, as observed in the laboratory at 30°C will not occur in the field. Under field conditions, flies will have an increase in activity from early morning, as observed on day 2 and 3 at 24°C in the laboratory, till the temperature becomes unfavourable. Activity will then be reduced till the temperatures are favourable again. This will result in two peaks, as is observed in the field.

The results seem to indicate that the basic patterns of flies from the two areas are slightly different. It also indicates that flies from the two areas are adapted to different optimal conditions. These two differences seem to have a genetical basis.

Genetic variation

In last year's Annual Report (1978), it was reported that three enzymes were found which showed variation:

leucin amino peptidase (LAP), at the LAP–3 locus, aldehyde oxidase (AO), and non-specific esterases (EST) at the EST–1 locus. In total 8 populations of *G. pallidipes* have now been studied for these three enzymes: Mwalewa (South Coast), Shimba Hills (South Coast), Galana Ranch (North Coast), Kibwezi forest, Meru National Park, Nkruman (Rift Valley), Lambwe Valley (Nyanza), and Sigor (N. of the Cherangani Hills).

Table 2 shows the allele frequencies of the three enzymes for all 8 populations. The results can be summarized as follows:

- The genotype frequencies of the three enzymes followed the Hardy Weinberg law in all populations, with exception of AO in Nkruman.
- The frequencies of the two alleles which were found at the EST–1 locus did not differ much in all populations.
- The 1.10 allele at the LAP–3 locus was found in low frequencies only. In the Kibwezi population it was found three times, and in Nkruman and Sigor once, as heterozygotes only. It was missing in other populations. The 0.95 allele was found in a frequency of 6% in Kibwezi, 4% in Mwalewa and 1% or lower in all other populations. The 1.05 allele was found in frequencies from 4 to 7% but was more frequently found in Kibwezi and Mwalewa (both 16%).
- The 0.95 allele at the AO locus was missing in the three coastal populations and also in Sigor. The 1.05 allele occurred in the highest frequency in Nkruman and Lambwe Valley (25 and 24% respectively), and in the lowest frequencies in the coastal populations (11% or lower).
- AO showed some results which were difficult to explain. The variation in an enzyme is supposed to be the same for sexes, and this was indeed found for EST and LAP. This was also true for AO in Kibwezi, Sigor and Galana. It was however, not true for the other 5 populations. In Meru, males showed a significant ($p < 0.01$) higher amount of variation in this enzyme than females, while in Shimba Hills, Mwalewa, Nkruman, and Lambwe Valley, females showed a significant ($P < 0.01$) higher amount of variation than males. In Meru, Shimba Hills and Mwalewa, the frequency of the genotypes of both sexes separately, fitted the Hardy Weinberg law. In Nkruman and Lambwe Valley, the frequency of the genotypes in males also fitted the Hardy Weinberg law, but the females did not. Both populations had too many heterozygotes.

Table 2. Allele frequencies of the loci of three enzymes, which showed variation, in 8 populations of G. *pallidipes* n = number of tested flies

	EST			AO				LAP				
	n	0.95	1.00	n	0.95	1.00	1.05	n	0.95	1.00	1.05	1.10
Mwalewa	163	0.05	0.95	200	—	0.98	0.02	224	0.04	0.80	0.16	—
Shimba Hills	299	0.02	0.98	314	—	0.89	0.11	299	0.01	0.93	0.06	—
Galana Ranch	455	0.03	0.97	455	—	0.89	0.11	455	0.01	0.94	0.05	—
Kibwezi Forest	208	0.08	0.92	215	0.03	0.80	0.17	200	0.06	0.78	0.16	0.01
Meru National Park	415	0.03	0.97	430	0.003	0.83	0.17	430	0·003	0.95	0.04	—
Nkruman	542	0.06	0.94	660	0.05	0.70	0.25	654	0.01	0.92	0.07	0.001
Lambwe Valley	298	0.01	0.99	300	0.01	0.75	0.24	300	0.002	0.98	0.02	—
Sigor	240	0.03	0.97	270	—	0.88	0.12	240	0.002	0.93	0.06	0.002

It is not yet possible to explain these results with the available data. However, AO is the enzyme with the largest differences between the populations. This might suggest that this enzyme is reacting most strongly on differences in selection pressure.

The results obtained in these studies might suggest that several populations of *G. pallidipes* can be distinguished in Kenya. The coastal population seemed to be isolated from the inland populations. Some differences were found within the coastal population, suggesting that some sub-populations might have arisen.

Nkruman and Lambwe Valley might belong to one population, together with the population from the Masai Mara. However, due to changes in the habitat, partly by human settlements, the three areas now seem to be isolated.

The Sigor population seemed to be isolated from all other populations. Kibwezi and Meru might have belonged to the same population, but the differences which were found between the two populations suggested that they had become isolated for some time.

Although several populations or population groups could be distinguished, no information on the degree of isolation and the type of barriers which might exist between the populations, have been obtained yet. Lack of information on the vegetation and rainfall before this century, makes it difficult to get an idea of the duration or existence of possible barriers. The results however, have undoubtedly confirmed the existence of genetic variation and population diversity in populations of *G. pallidipes*.

Tsetse ecology on the Kenya coast

W. F. Snow

A programme to study aspects of the population biology of the tsetse fly, *Glossina pallidipes*, on the south Kenya Coast, using the facilities of the ICIPE coastal field station, began in 1978. Topics which are receiving particular attention include population fluctuations in relation to adult mortality patterns indicated by age-grading dissections of samples of female flies, comparison of the eco-behavioural characteristics of *G. pallidipes* at different localities on the basis of population size mortality patterns and the reproductive and feeding strategies of both male and female flies. Data on the general distribution and ecology of tsetse are also being collected to provide a basis on which to advise the Kenya Veterinary Department on aspects of tsetse control.

Monthly samples, usin; Challier/Laveissiere traps have been taken at Muhaka since mid 1978. This is an isolated area of forest where a medium-density population of *G. pallidipes* occurs and cattle and wild pig form the main blood-source for these flies. In August 1979, two other localities, previously investigated on a less regular basis, were adopted for a similar monthly sampling route. In Shimba Hills National Reserve very large populations of *G. pallidipes* are present with abundant wild hosts including antelope, buffalo, pig and elephant although domestic animals are absent. A very low density population of *G. pallidipes* is present at Ukunda Veterinary Research Station with a few cattle, sheep, goats and camels, although wild pigs are probably also common in the area. Occasional observations continue to be made on a farm at Diani and in Mwalewa Forest near the Tanzania border.

Fly density is assessed in relative terms from the catch per trap per day. The age structure of the female component of samples is evaluated using ovarian age-grading techniques. From the position of the largest ovariole and the presence or absence of scarring on the follicular stalks, indicating previous ovulations, 8 age categories can be recognised. Assuming a logarithmic death rate, the age-composition of the sample can be estimated. The slope of the survivorship curve is used as an index of the mortality of the sample. From the length of the largest of the four ovarioles, the relative age of each female within its current reproductive cycle can be estimated.

It is too early to draw firm conclusions regarding seasonal changes in the numbers of *G. pallidipes* from the data obtained so far at Muhaka. However, as regards population fluctuations in general, tsetse appear to live longer when the population is increasing than when it is decreasing. Mortality indices are significantly higher in an apparently declining population. This observation indicates that, to a large extent, population fluctuations can be explained on the basis of adult mortality patterns alone. Although climatic conditions are equable on the coast a relationship between population change in *G. pallidipes* and rainfall during the preceding month is becoming apparent. With precipitation of less than 70mm the tsetse population declines with high adult mortality perhaps related to low humidities. Between 70 and 200mm the population usually increases, but with higher rainfall tsetse numbers again tend to decline. Direct observations on pupal ecology and mortality for *G. pallidipes* are exceedingly difficult. However, potential rates of increase for a population can be computed and comparison with observed changes in numbers may enable pupal mortality to be estimated.

The comparison of the eco-behavioural characteristics of different populations of *G. pallidipes* has concentrated on the relative density of tsetse and mortality patterns. There is no clear relationship between population size and mortality, although the absence of high population densities with high mortality indices is very significant. Mortality patterns and population size characteristics may be typical of a particular population. These may be related to habitat type and area, host availability and climatic differences although these latter are very marginal between the south coast localities. The detailed observations from Shimba Hills and Ukunda for comparison with the Muhaka data should contribute much to this aspect of the study.

Measuring the length of the largest of the four ovarioles indicates the relative age of each female within its current reproductive cycle. When nulliparous females, in their first cycle, are considered, 2–4 and 6–8 day-old groups predominate in the traps (Fig. 2). The former are all virgin and the majority of the latter are inseminated, indicating that mating usually takes place between 4 and 6 days. The results also indicate 2 peaks of responsiveness to the traps in nulliparous flies. If the tsetse mistake a trap for a host, and this remains to be established by catches from bait animals, then flies may take 2 blood-meals before the first ovulation. The situation with older flies is less clear although they may take 3 or 4 meals during each cycle of larval development.

As regards long-term startegy it is acknowledged that in terms of pest control in Africa the results of this project must be generally applicable under conditions where facilities and staff training may be less than ideal. The project will develop themes including:—

- —The basis of an understanding of the population dynamics of tsetse populations. It is intended that this will have predictive value in both natural and control situations.
- —New and simplified approaches to the evaluation and monitoring of tsetse populations. These would have direct application in the planning and assessment of conventional and novel control methods.
- —The development of new, environmentally sound, integrated control strategies with emphasis on low cost and simple technology.

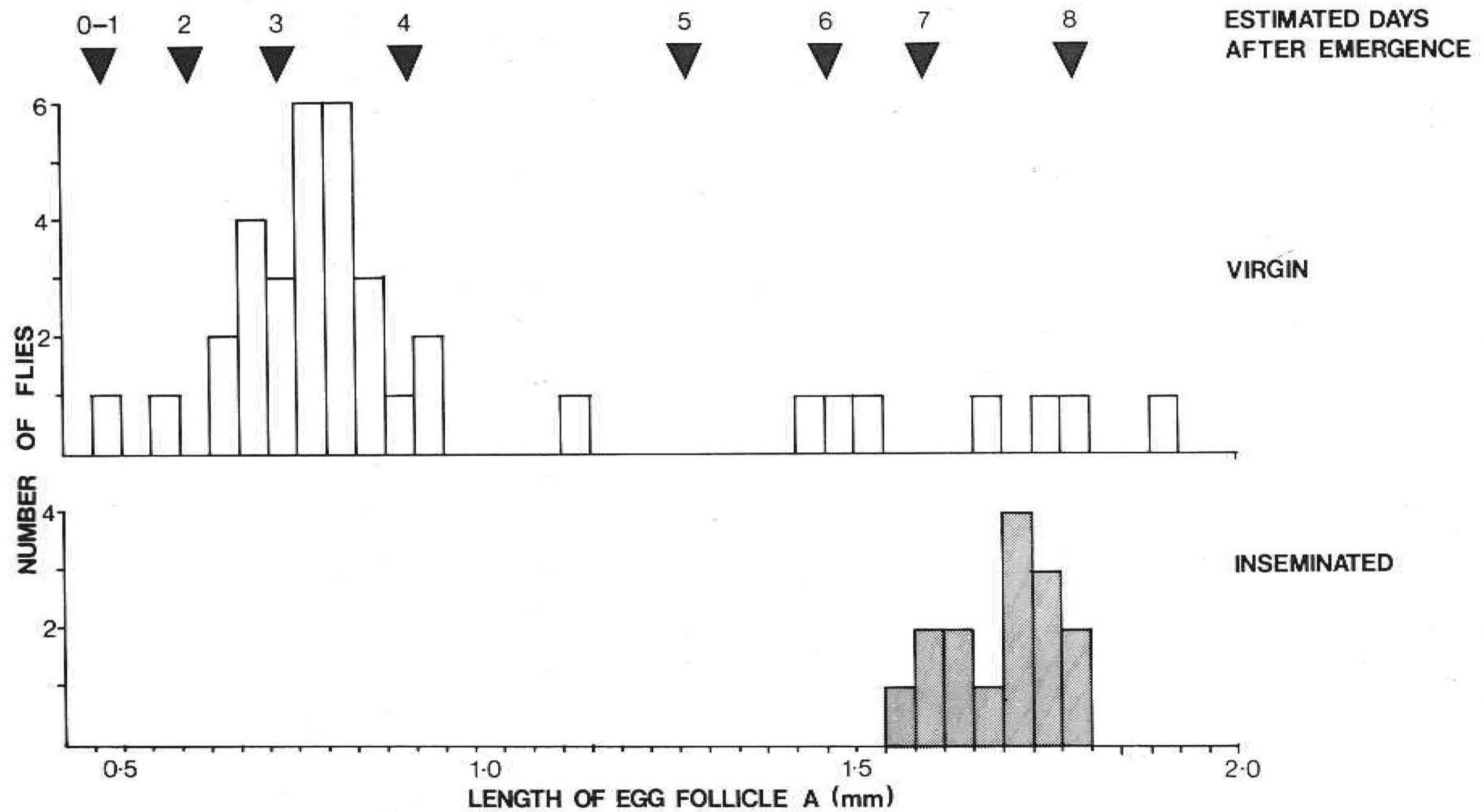


Fig 2: Length of the first egg follicle to develop in female *G. pallidipes* related to insemination and estimated age.

The ecology of the tsetse fly *Glossina pallidipes* Aust. in the Lambwe Valley, South Nyanza.

D. A. Turner

A programme on the ecology and behaviour of *Glossina pallidipes* was started in August 1979 in the Lambwe Valley Game Reserve. This is an area of considerable epidemiological interest where, in addition to livestock trypanosomiasis, low grade transmission of *Trypanosoma rhodesiense* persists in settlements around the periphery of the Reserve.

Samples of flies collected monthly in biconical traps are being used to study the dynamics of populations in relation to climate, vegetation and food supply. For comparative purposes two localities are presently under study: a typical habitat of dense thicket at the bottom of the valley (Ruma thicket); and an atypical habitat afforded by an exotic coniferous plantation on the eastern escarpment of the valley. It is intended to include a third locality characterised by *Acacia* woodland, situated intermediately. Tsetse populations in these habitats are supported by an abundant wild animal population.

Results so far indicate an overall high density of fly in each locality, but with considerable trapping site variation and daily variation in catch. Sexes are caught in roughly equal proportion. The age structure of the female component of the population is being evaluated by the ovarian age-grading method for determination of mortality patterns. Wing fray measurements have been found to give a useful rough indication of the probable age category prior to dissection. Here, the data indicate that the catch is deficient in young (nulliparous) females throughout, and that the thicket-dwelling population contains a much larger proportion of older females (categories 4 and above) than that of the coniferous plantation.

Blood meal samples are being collected for serological identification and eventual determination of host preferences and availability.

Preliminary marking/release/recapture experiments have been carried out in a relatively isolated area of conifer plantation from which an initial assessment is being made of fly density, dispersal patterns, diel activity cycle and feeding interval.

Routine examination of flies has revealed a low level of parasitism by an ecto-parasitic mite, which is being identified. A possible pathogenic agent has also been discovered. This appears as a black scale firmly embedded in the soft cuticle of the abdominal undersurface. Liaison has been made with the Tsetse Officer, Dept. of Vet. Services, Sindo, to test the efficacy of the biconical trap against *Glossina fuscipes fuscipes* along the lake shore. Preliminary results indicate that the trap is efficient for sampling this species, and will prove to be a useful aid to survey.

Mention has been made of an atypical habitat of *G. pallidipes* afforded by coniferous plantation. This is the first recorded instance of the adaptation of tsetse to this type of vegetation. In view of the possible threat of extended tsetse distribution from forestation practices, attempts will be made to survey other coniferous plantations contiguous to tsetse belts. Two areas, the Shimba Hills on the Coast and a plantation near a *G. f. fuscipes* habitat at Oyugis in South Nyanza, have been identified for future survey.

Relative efficiency of some mechanical traps used in the study of *Glossina Pallidipes*

M. L. A. Owaga

Challier-Lavessiere's biconical trap was compared with Langridge's boxscreen, and Moloo's awning screen skirt, for efficiency in catching *Glossina pallidipes*. The parameters examined included total yields, mean age of male flies, hunger stage and sex ratios.

The biconical trap which had not been tried for East African species was originally developed in West Africa for the riverine species of *Glossina*. It had proved particularly efficient for sampling *G. palpalis gambiensis* Vanderplank. In this study the performance of the biconical trap was compared to that of Langridge's box screen (LBS) in Kibwezi forest, Kenya; and Moloo's awning screen skirt (ASS) in Nkruman escarpment in the Rift Valley, some 360 km from Kibwezi. These two kinds of screens had earlier been confirmed as the most efficient in the respective areas.

Three versions of the biconical trap were used; one with a sky-blue lower cone (BBs), another with a dark-blue lower cone (BBd) and a third with white lower cone (WB). The top cone was always white. Challier had found in Upper Volta that the colour contrast between the lower and upper cones of that trap has an effect on its relative attraction for *G. palpalis*.

The experiment was done in three localities about 250m apart. At each locality a set of three traps, one WB, one BBs and one LBS were placed about fifty metres apart. (In Nkruman ASS replaced LBS, and during the second half of the study BBd replaced BBs). The traps were emptied every 24 hours and rotated from site to site. On completion of the rotation a two week interval was observed to enable the fly population to rebuild. Total yields as well as sex ratios were recorded; the mean age of male flies caught by each kind of trap was determined by the wing fray method. They were placed in three categories, namely G1, G2, and G3.

Since male tsetse flies are more active than female ones, their wings tend to fray at a a faster rate. The amount of fray can directly be related to the age of the fly. G1 included very young flies of wing fray one (no damage), G2 comprised young and middle age flies of wing fray two and three, and G3 was composed

of old and very old flies of wing fray four, five and six. Age estimate of female flies was done by the ovarian method.

The results of trap yields in Kibwezi and Nkruman for eleven and six trapping periods respectively are given in Table 3, which also shows sex ratios. Mean age estimates for males are shown in Figure 3. In Kibwezi forest the BBs gave significantly higher yields than both the LBS and WB ($P < 0.001$). The WB gave the lowest yields. The BBs also caught the highest number of female flies. In Nkruman the ASS performed better than a sky blue biconical (BBs) but when the colour was changed to dark blue (BBd) the latter gave higher but not significantly higher, yields. The WB gave the lowest yields. All traps in Nkruman gave higher yields of males than females, but the difference was not statistically significant.

Table 3. Total yields and sex ratios of *Glossina pallidipes* from different traps in Kibwezi and Nkruman

Locality		No. of Females	No. of Males	Total	% Females
	LBS	207	150	357	58.0*
Kibwezi	BBs	268	220	488	54.9
	WB	183	128	311	59.0*
Nkruman	ASS	1,119	1,179	2,298	47.8
	BBs	300	338	638 } 1505	47.0
	BBd	397	470	867 } 1505	45.8
	WB	268	273	541	49.5

*p<0.05

s — sky blue

d — dark blue

LBS, BBS ← P<0.001
WB, BBs ← P<0.01
WB, LBS ← P<0.05
ASS, WB ← P<0.001

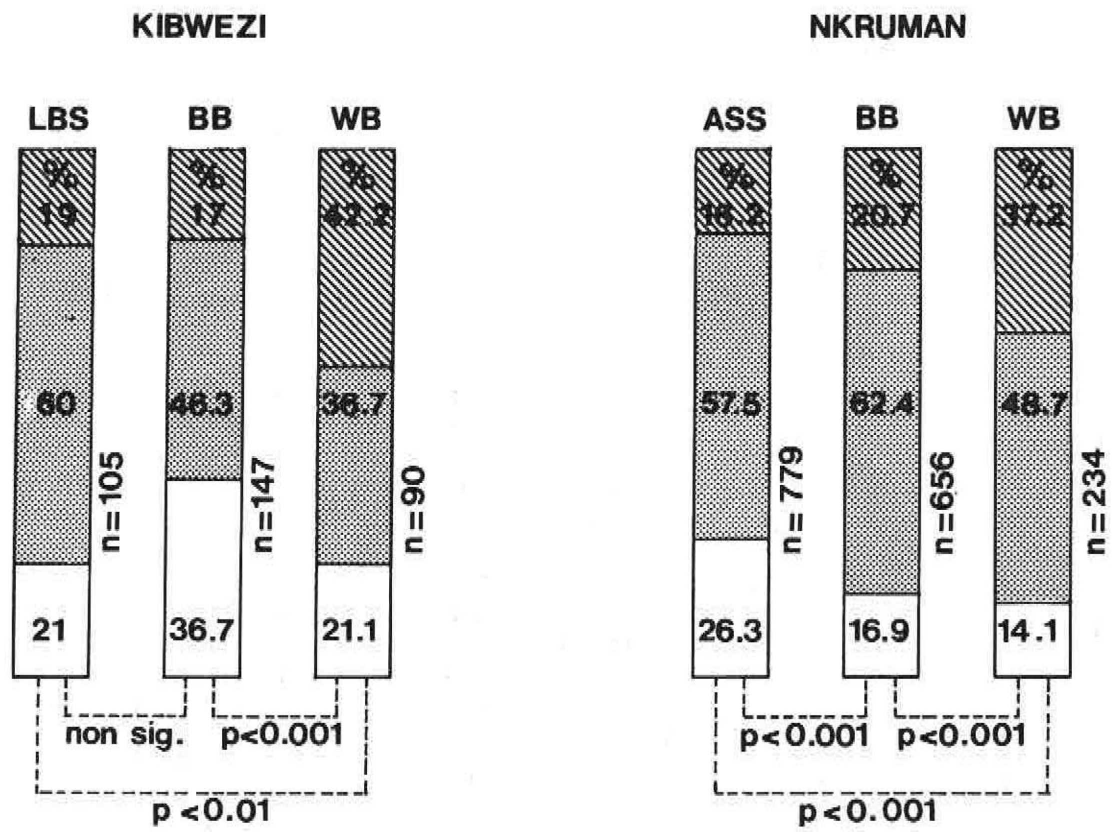
No significant difference in mean age was apparent between LBS and BBs traps in Kibwezi for males, but the difference between WB and BBs was highly significant ($P < 0.001$), WB catching the highest proportion of old flies. The mean age of males caught in the LBS and WB was also significantly different ($P < 0.01$) LBS had a higher proportion of young flies. In Nkruman the difference in mean age between males caught in the different traps was highly significant ($P < 0.001$). WB gave the highest proportion of old flies although the number was smaller than in the other traps. BBs and BBd had the highest number in G3 but this was composed mainly of flies of wing fray 4 while those caught in WB were mainly of wing fray 5 and 6; ASS had the highest number of young flies in category G1. Similarly the WB caught the highest proportion of females of old physiological age while ASS caught the highest proportion of young females.

The results indicate that trap yields depend on the areas and may vary from area to area even if the same individual trap is used. However, the BBd showed superiority over the LBs in Kibwezi and slightly better performance than the ASS in Nkruman. Sex ratios also differed according to areas. Since traps catch only the active section of the population sex ratios can indicate differences in activity pattern between various populations. The white biconical gave a 3:2 female: male ratio in Kibwezi and only 1:1 ratio in Nkruman. In Kibwezi the female number was significantly higher than the male number ($P < 0.05$).

In both areas the traps caught only hungry flies. It seems that these stationary baits attract hungry rather than deplete or gorged flies.

Unlike the LBS and the ASS the biconical trap is not animal shaped. It has a number of advantages over the other two. It is collapsible and light in weight, and thus easier to transport and store. It is the only mechanical trap which offers some physical restraint on the departure of a fly that has landed on its dark attractive part. It offers a greater light source from the top cone, and it can be set up in a few minutes. A number of them can be used at a time with minimal manpower. It is, therefore, preferable for most field studies. However, like the LBS and ASS it does mot restrain all the flies that land on it.

FIG.3 AGE ESTIMATES OF MALE G. PALLIDIPES FROM DIFFERENT TRAPS



AGE GROUPS

G1

G2

G3

Research Advisors
Professor W. S. Bowers (1977)
Professor J. H. Law (1977)
Scientists in Residence
Dr. J. D. O'Connor (1979)
Dr. F. J. Kezdy (1979)
Research Staff
Mr. A. Chapya (1974) Principal Technician
Mr. I. Jondiko (1979) Graduate Research Scholar
Mr. N. Juma (1974) Junior Technician
Dr. P. McDowell (1979) Postdoctoral Research Fellow
Dr. D. Otieno (1979) Postdoctoral Research Fellow
Mrs. M. Vundla (1975) Assistant Scientific Officer
Dr. D. Whitehead (1979) Senior Research Scientist
Collaborator
Dr. T. Gebreyesus

Chemical Identification of Termite Trail Pheromones

Phillip G. McDowell

A new project has been initiated with the objective of chemically identifying the trail pheromone components of termites. The species chosen for initial investigation was *Macrotermes michaelseni* (Sjöstedt) (formerly classified as *Macrotermes near subhyalinus*) since the trail pheromone was known to be stable, and some of the trail following behaviour was known.

Previous bioassay work has shown that the hexane extracts of the major workers of *M. michaelseni* (whole body extracts taken at —16°C for 24 hrs.) elicit trail—following behaviour in workers in a figure eight bioassay. Whole body extracts were therefore used as a source of material for the isolation of components for identification. The source of the pheromone had not been confirmed, although it was suspected that the sternal gland was the source, as has been shown for a number of other termites.

In the first instance analysis of the extracts was performed by gas chromatography (GC) on polar and non-polar phases. The longevity of the natural trails was observed to be very high, a well established trail being active for up to 7 hours. It was obvious, therefore, that the pheromone components were of rather low volatility. This being the case, a 3% OV—101 (non-polar) GC column was used for the first analyses. A temperature programmed run from 220°C to 290°C was employed and chromatograms showed the presence of a mixture of components as shown in Figure 1.

Chromatograms programmed from 70°C confirmed the absence of more volatile components. As stated above, it has been demonstrated in a number of other termites that the sternal gland is the source of the trail pheromone. *M. michaelseni* possesses a well developed sternal gland in each caste. Glands were therefore dissected from major workers and extracted in hexane at —16°C for GC analysis. Analyses of these gland extracts were similar in constitution to the whole body extracts except for small variations in the relative amounts of the individual components. Bioassay work showed that these gland extracts were extremely active in trail following. Further analysis of the gland content was achieved by the use of a solid sample gas chromatography (SSGC) technique which allows the direct injection of volatiles from a gland without the use of solvent. Volatiles from between 2 and 6 glands were injected by this technique and the resultant chromatograms indicated the presence of components in the gland similar to those in the gland extract and the whole body extract. The quantities per gland, however, were very small and the chromatograms from the SSGC technique were not as good as those from the extracts.

Having ascertained that there were compounds in the sternal glands and that sternal gland extracts were both trail active and similar to whole body extracts, the problem of isolating and identifying components was undertaken.

Before fractionation a whole body extract was subjected to combined gas chromatography mass spectrometry (GC–MS) in order to obtain some idea of the nature of the compounds being dealt with.

The mass spectra obtained indicated components of a long-chain hydrocarbon nature of chain length C_{23} and over. Microscale reactions such as bromination indicated the presence of both saturated and unsaturated compounds. Further investigation of the extract components required fractionation. This was achieved by preparative GC and to this end an all-glass micro-preparative effluent splitter and collection system was designed and constructed for a Packard 428 GC. A total of eight fractions were collected from a 3% OV–101 column (figure 1 shows the 8 preparative sections). Further identification work is now being carried out on the individual components of the extract.

Bioassay work on the separated fractions has so far proven inconclusive, but testing of the fractions and individual components continues.

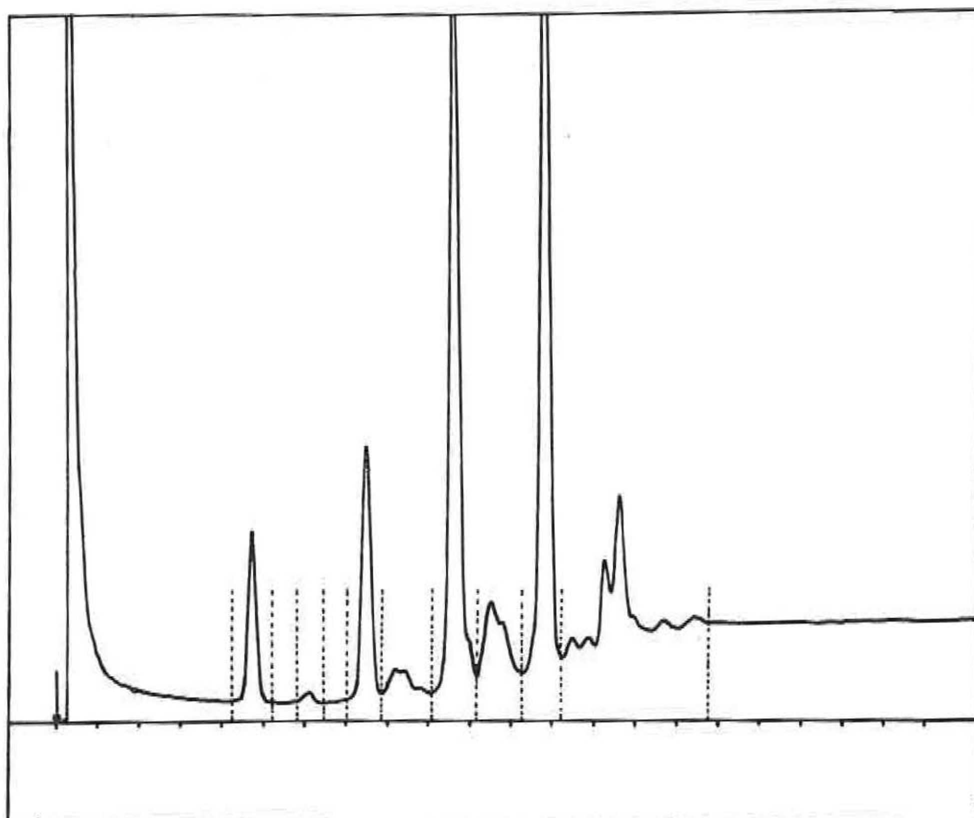


Figure 1. Gas chromatogram of *Macrotermes michaelseni* major workers extact. Stationary phase: 3% OV-101: Temperature programme: 220°C (for 10 min.) to 290°C at 4°C/min. rise rate. The preparative fractions taken are indicated by the sections marked 1 to 8.

Chemical Studies on Excreta of the Soft Tick *Ornithodoros porcinus porcinus*

D. A. Otieno

Introduction

Previous work (ICIPE Annual Report, 1977) has shown that excreta of *O. p. porcinus*, *Argas persicus* and other soft ticks contain assembly pheromone(s) which have some potential as bait in development of tick control agents. Investigations on the chemical identity of these pheromone(s) have been attempted previously (ICIPE Annual Report, 1974) without success. During the period under review, further studies have been undertaken on the excreta of the soft tick *O. p. porcinus* in order to gain insight into the nature and chemical identity of these compounds. A summary of the progress made is given in this report.

Materials and Methods

Two batches of 100 fully-fed adult ticks from laboratory colonies were used as sources of excreta. One batch was thoroughly extracted with distilled water. The other batch was exhaustively washed with physiological saline solution. Both extracts were then freeze-dried. For analysis, aliquots of the excreta were weighed and dissolved in the eluent of high performance liquid chromatography (HPLC) and filtered using a syringe-adapted metricel filter of 1.0μ pore size. Separation of the components of the extracts was achieved by HPLC using a Zipax SCX column and nitric acid: ammonium nitrate buffer (pH 1.4) as the mobile phase. The HPLC eluent was completely degassed prior to use. All experiments were performed at room temperature 21±2°C). The HPLC used consisted of a Waters Associates ALC 202 liquid chromatograph equipped with a differential ultraviolet (UV) detector (1 cm. path length) operated at 254 nm. Monitoring the eluent for UV absorption and integrating the areas of the individual peaks proved to be a rapid and accurate technique for quantitating the many components of the extracts. The eluted peaks were recorded and integrated using a Kipp and Zonen Micrograph BD 5 recorder and a BC1 integrator. Identification of the individual components of the extracts was achieved through mass spectrometry and UV absorption spectra, and by comparison of their chromatographic characteristics with those of authentic samples.

Results

Figure 2a shows the separation of the constituents of the aqueous extracts. The UV elution pattern of a sample of saline washings is depicted in Figure 2b. The compounds eluted fourth and fifth were identified as guanine and adenine respectively on the basis of mixed

HPLC, UV and mass spectral evidence. UV and mixed HPLC data suggested that the product eluting first is probably an inorganic salt of either sodium or potassium, but unambiguous identification of this fraction was not estab.ished. Bioassay data (see Livestock Tick Research section of this Report) showed that guanine and the fractions eluting second and third. were the most active eluates from the HPLC.

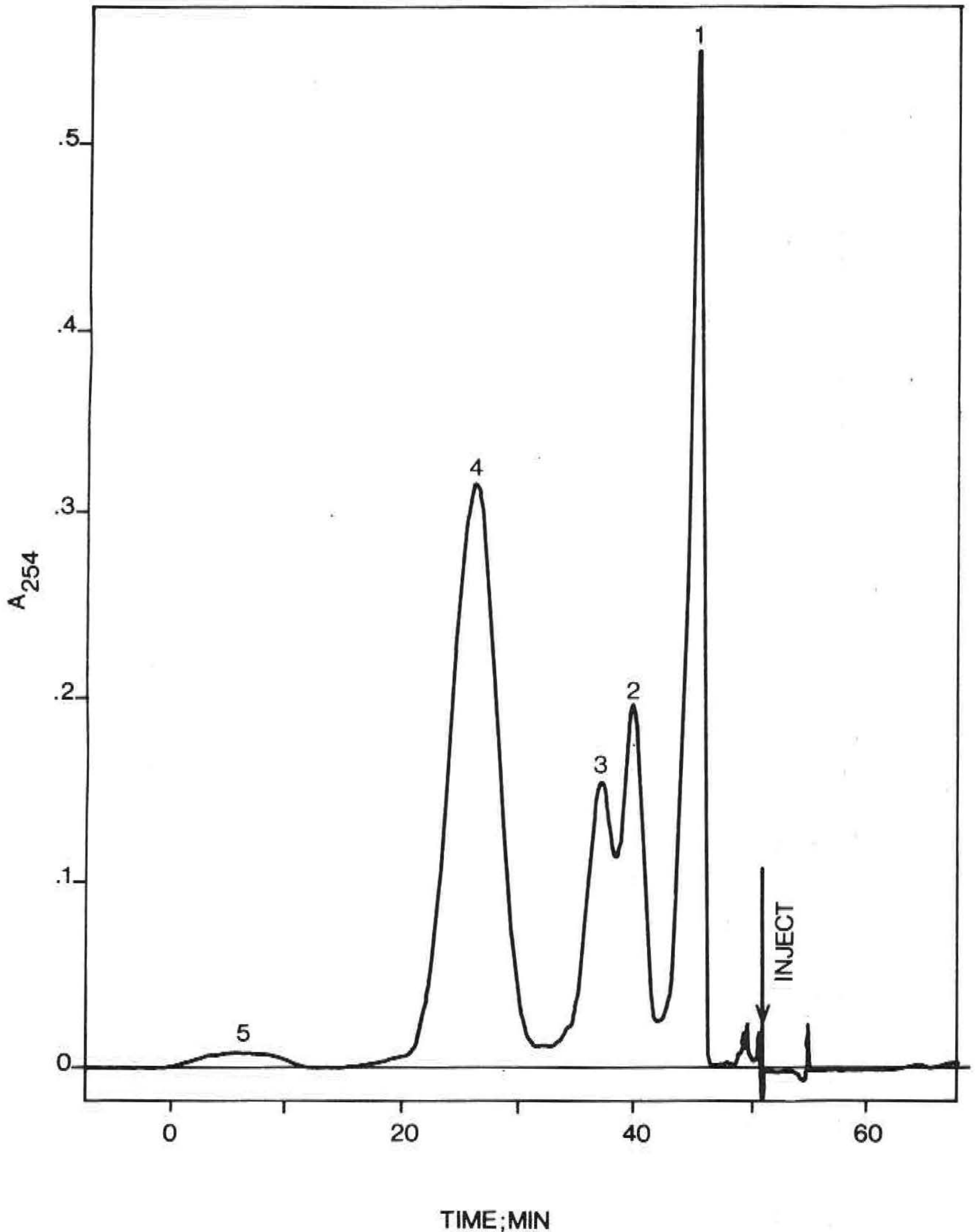


Figure 2a. HPLC separation of the components of aqueous extracts of excreta of the soft ticks *O. p. porcinus*.

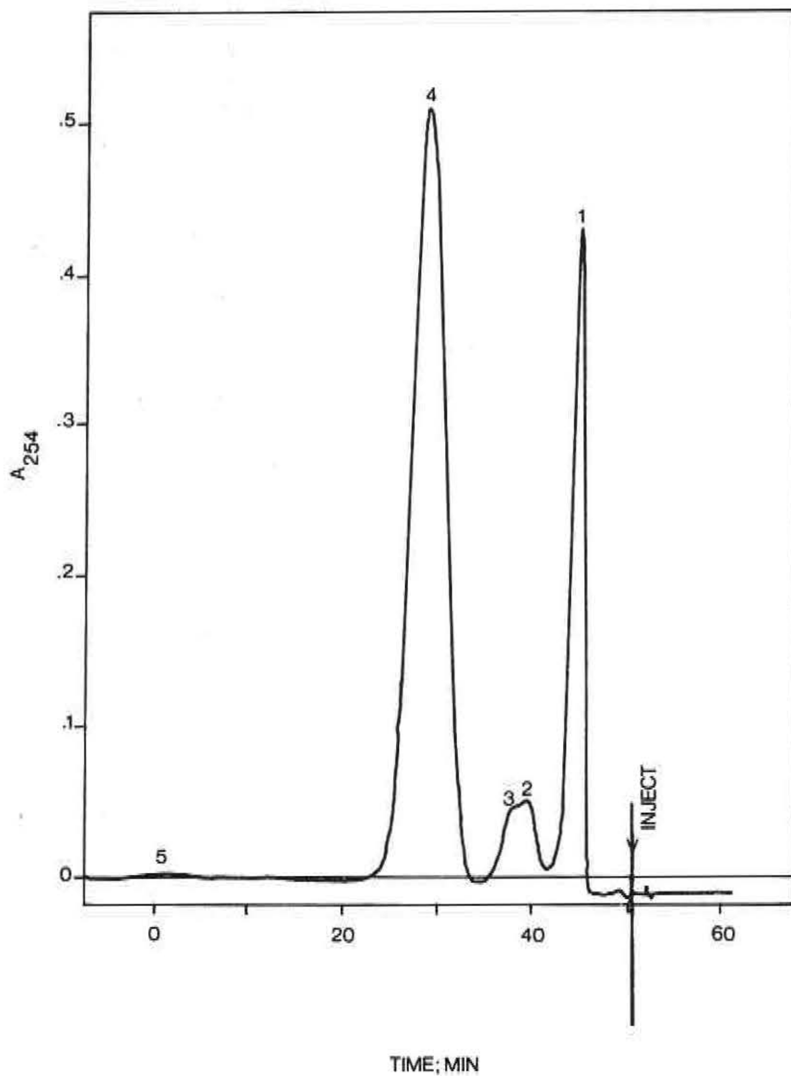


Figure 2b. HPLC separation of the constituents of the saline extracts of excreta of *O. p. porcinus*. Operating conditions in both cases: Pump pressure: 700 psi; Temperature: Ambient; Flowrate: *ca.*1.5ml min^{-1}; Detector: UV (254nm). 0.08 AUFS. Peak Identity: 1 — inorganic salts?, 2, 3 = unidentified compounds 4 = Guanine and 5 = Adenine.

Integration of the UV elution peaks revealed that guanine, the compound eluted fourth in HPLC was the predominant (>95%) component of excreta of the soft tick *O. p. porcinus*. As depicted in Figure 2b, washing the ticks with physiological saline, aqueous amonia (0.143M) and aqueous hydrochloric acid (0.1M) solutions led to extracts containing predominantly guanine and only small quantities of the other components. Adenine was detected in these extracts only at sensitivities higher than that shown in either Figure 2a or Figure 2b, and in all the cases, the amount detected was negligible.

Discussion

When eluants from HPLC were pooled, they were found to display aggregation activity closely similar to that of unfractionated extract. Moreover, the refractive index detector revealed the same components of the extracts as did the UV. detector. These two experiments show that the HPLC column did not retain any vital ingredient of the extract. Therefore taken together, information summarised in this report shows that the dominant and most active ingredient of excreta of the soft tick *O. p. porcinus* is guanine. Work is now in progress to establish the identity of the two active ingredients eluted second and third in the HPLC, and to establish the actual chemical form in which guanine displays activity. Since *A. persicus*, the chicken tick was used for all bioassay tests (it responds much more positively than other species of soft ticks) the possibility that purine or pyrimidine bases occur in chicken exreta, to which *A. persicus* ticks might be attracted, is also being examined.

Isolation of Ingredients of Pyrethrum Extract

D. A. Otieno
I. Jondiko,
N. Juma,
P. G. McDowell and
F. J. Kezdy

Introduction

One of the many beneficial properties of pyrethrum extract is that it is a powerful insect repellent. However, the chemical identity of the compound(s) responsible for this property is not known. Knowledge of the identity of the insect repellent in pyrethrum extract would enhance its economic value. Systematic investigations were started on the extract in order to discover this repellent. These investigations required a technique for isolating pure pyrethrum insecticides and the many other ingredients of pyrethrum extract in reasonable quantities for bioassay tests.

We have now developed a high performance liquid chromatography (HPLC) method suitable for achieving this objective. The details of the method are summarized in this report.

Experimental Methods

The HPLC apparatus used consisted of a Du Pont Instruments 848 pump module with a calibrated 10μl injector, a Water Associates Differential Refractometer R401 and a Water Associates Ultraviolet (UV) detector (1 cm path length cell) operated at 254 nm. The individual eluted peaks were recorded and integrated using a Kipp and Zonen Micrograph BD5 recorder and a BCI integrator. Successful separation of the six pyrethrin esters was achieved with a double μPorasil column (10μ particle size 0.4cm, i.d.x 30cm) using a 1:1 mixture of anhydrous and water saturated dichloromethane as the mobile phase. The individual pyrethrin esters were identified through mass spectrometry and UV absorption spectra.

Results

Figure 3 shows the separation of the six pyrethrin esters. The corresponding mass spectra of these compounds are depicted in Figure 4. Integration of the UV peaks (Fig. 3) afforded data (PyIs 35.4%, PyIIs 23.95%; Total 59.4%) which are in excellent agreement with those (PyIs 35.52%; PyIIs 24.69%; Total Pys 60.21%) obtained by chemical analysis (performed

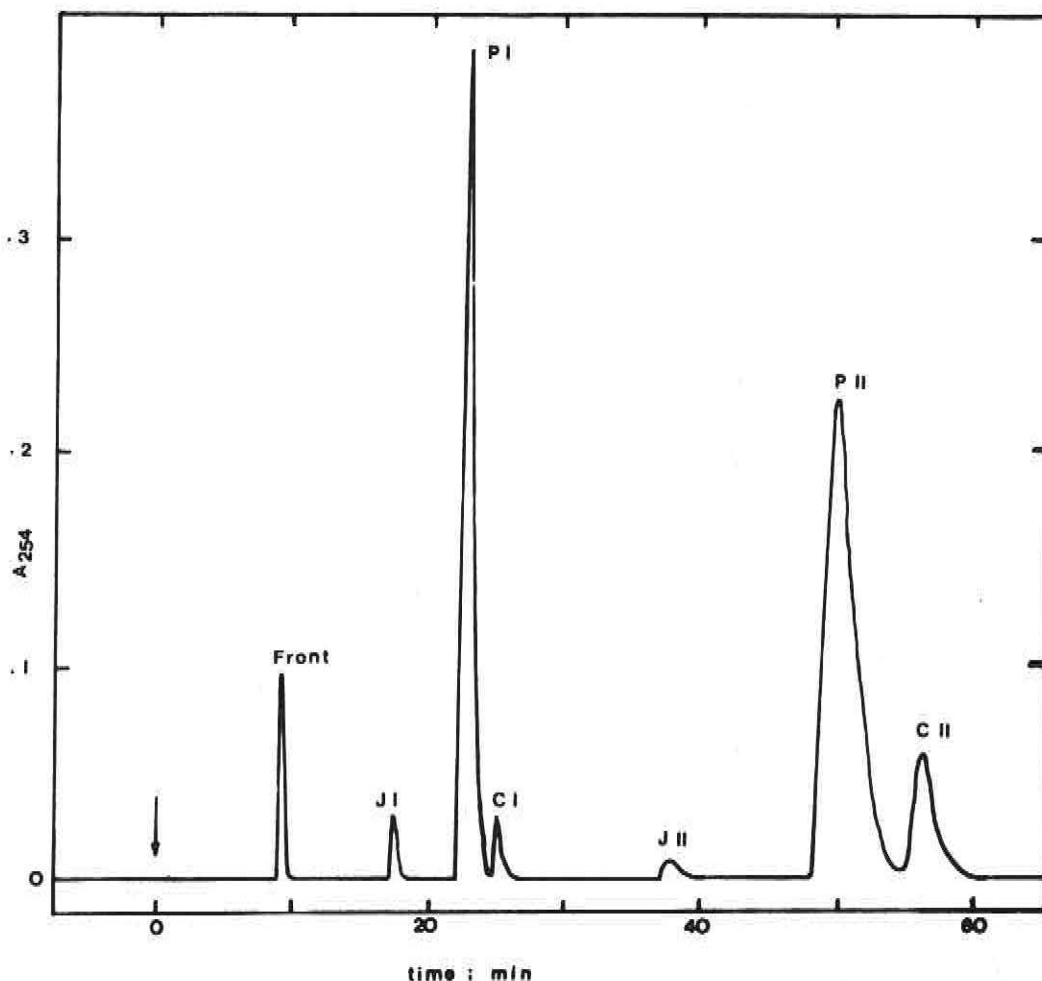


Figure 3. HPLC separation of the natural pyrethrins.

by the laboratories of the Pyrethrum Board of Kenya on the same extract). The refractive index (RI) detector showed the presence of several additional well-resolved non UV absorbing compounds in the extract.

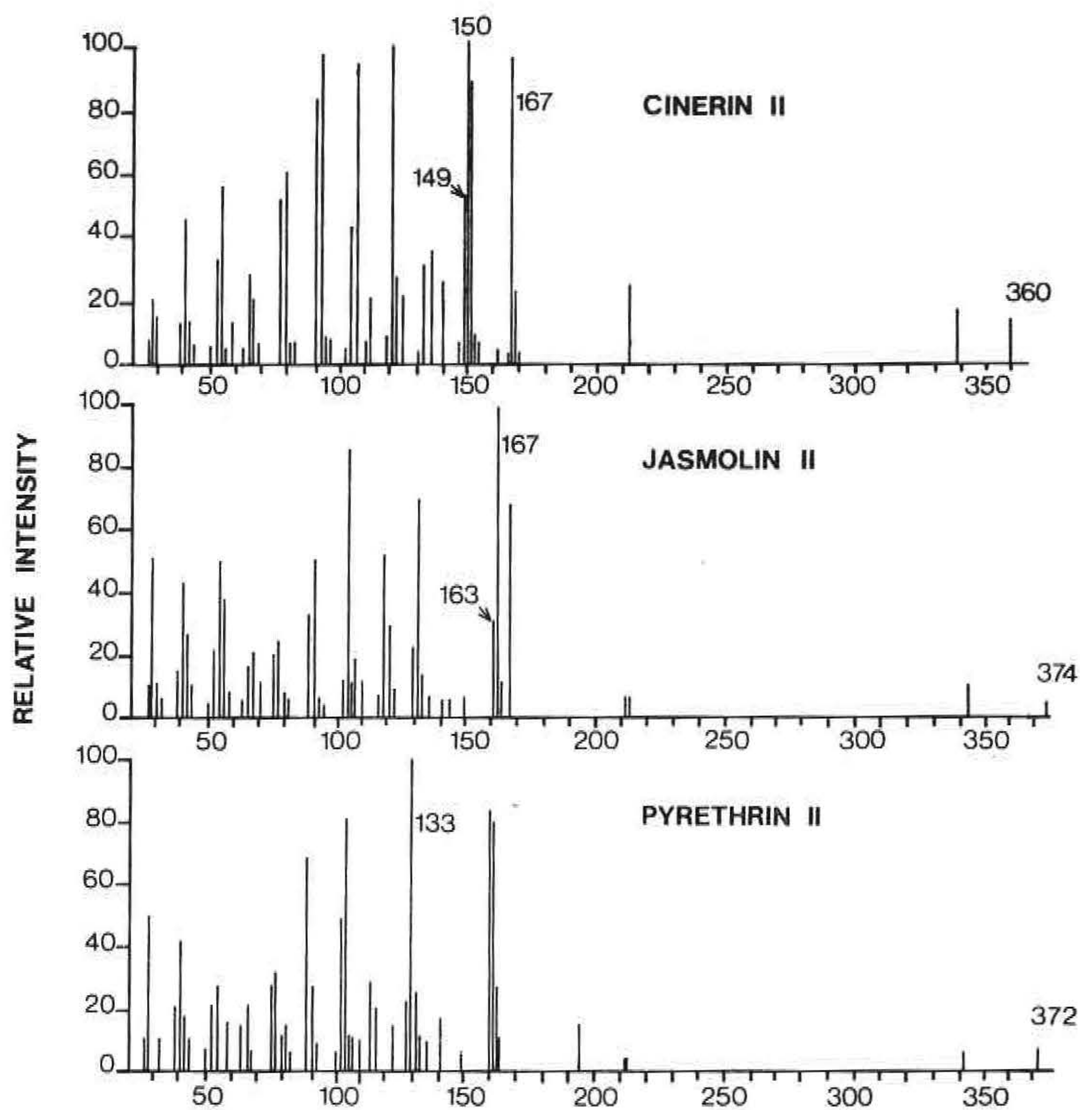


Figure 4. The mass spectra of the pyrethrin esters.

Conclusion

New methods of analysis of the natural pyrethrin insecticides are of benefit to the Pyrethrum Industry in Kenya where the existing analytical techniques are inadequate to monitor rapidly and reliably, the many industrial processes leading to marketable pyrethrum products. The HPLC method described above is not only suitable for isolating pure pyrethrin esters and the many additional components of the pyrethrum extract for further biological examination, but also satisfies this industrial analytical requirement. Work is now in progress to test each fraction from the HPLC in order to discover the repellent, and to characterise the hitherto unknown non-pyrethrin components of the extract.

Aknowledgements

We thank the Pyrethrum Board of Kenya for gifts of Pyrethrum extract.

Activity of Extracts of Bark of *Albizia coriaria* Against *Aedes aegypti* Mosquito Larvae

J. I. Jondiko and D. A. Otieno

Introduction

Albizia coriaria is a tall (30 m) leguminosae tree found in the riverine forests and grasslands in central, eastern and southern Africa. In Kenya, it grows wild in Nyanza, Rift Valley and Western Provinces and serves as a good source of building timber. Minced bark and leaves of the tree are used in some parts of Kenya, Uganda and Zaire to incapacitate fish before catching them for food. Further, the bark infusion has several other functions. In some regions of Kenya, for example, local people use it to treat menorrhagia, postpartum haemorrhage and threatened miscarriage whereas the root extract is used for the treatment of veneral diseases and sore eyes. In Katanga, Zaire, the bark and root infusion is used as a disinfectant and medicine for blennorrhoea.

On a chemical note, several natural products including glycosides, sapogenins, triterponoids tannins etc. have been isolated from several species of *Albizia* trees. We are, however, unaware of any corresponding work on *Albizia coriaria* species. In the course of our search for insecticides, larvicides and insect antifeedants from plant sources, we have examined methanol, acetone, chloroform and hexane extracts of the bark of *A. coriaria* for larvicidal and insecticidal activity. Below are the results of larvicidal tests done with these extracts using *A. aegypti* mosquito larvae.

Experimental methods

(a) Extraction procedure

Freshly collected bark of *A. coriaria* was chopped into small pieces which were dried for 10 days and ground to a fine powder (600 g). Portions of the powder (60 g) were soxhlet-extracted successively with 300 ml each of hexane (20 h), chloroform (60 h), acetone (30 h) and methanol (60 h). The hexane, chloroform and acetone extracts were each concentrated *in vacuo* at 35°C and the dried residues where dissolved in methanol and were each bioassayed without further treatment. In a similar manner, the methanol extract was concentrated *in vacuo* to 50 ml and bioassayed as such without further treatment.

(b) Bioassay tests

Bioassay solutions containing 3.35 and 0.22 mg ml^{-1} of the methanol extract gave percentage kill (of the third instar larvae of *A. aegypti*) values which were used as the upper and lower limits respectively in the subsequent bioassays.

It was predetermined that the biossay solutions were to be 100 ml each. Therefore, 10 ml of the concentrated methanol extract was diluted serially in such a manner that when 1 ml of each of the resulting five dilute solutions were diluted 100 times, the strengths of the bioassay solutions shown in Table 1 were obtained.

A suspension of 20 third instar larvae of *A. aegypti* in water (19 ml) was transferred into a mixture of the serially diluted solutions (1 ml) and water (80 ml) contained in a 250 ml beaker. One ml. of methanol instead of 1.0 ml of the dilute methanol extract, was used as a control. The beaker and contents were kept at room temperature for 24 hours after which period, the dead and moribund larvae were counted, pooled together and treated as affected (Table 1).

Results and Discussion

Larvicidal bioassay data, for the methanol extract are shown in Table 1. The relationship between concentration and the larvicidal effect of the extract is depicted in Table 2 and Figure 5.

Extracting the residue from the methanol extract with *n*-butanol and concentration of the *n*-butanol extracts in *vacuo* gave a solid with an apparently lower LD_{50} value than the one shown in Table 2, indicating that this is a reasonable step in purification. Hexane and chloroform extracts were found to be inactive while the acetone extract only showed slight activity.

Conclusion

The above data show that a methanol extract of *A. coriaria* has potent larvicidal principles which it is hoped to isolate and identify. The activity may be confined to a saponin with surfactant properties. Work is also in progress to establish whether or not the extract displays insecticidal or antifeedant activity.

Aknowledgements

We thank the Bioassay Research Unit for larvicidal tests.

FIGURE 5

Figure 5. The toxic effect of methanolic extract of bark of *Albizia coriaria* on late third instar larvae of *A. aegypti* at varying levels of concentration.

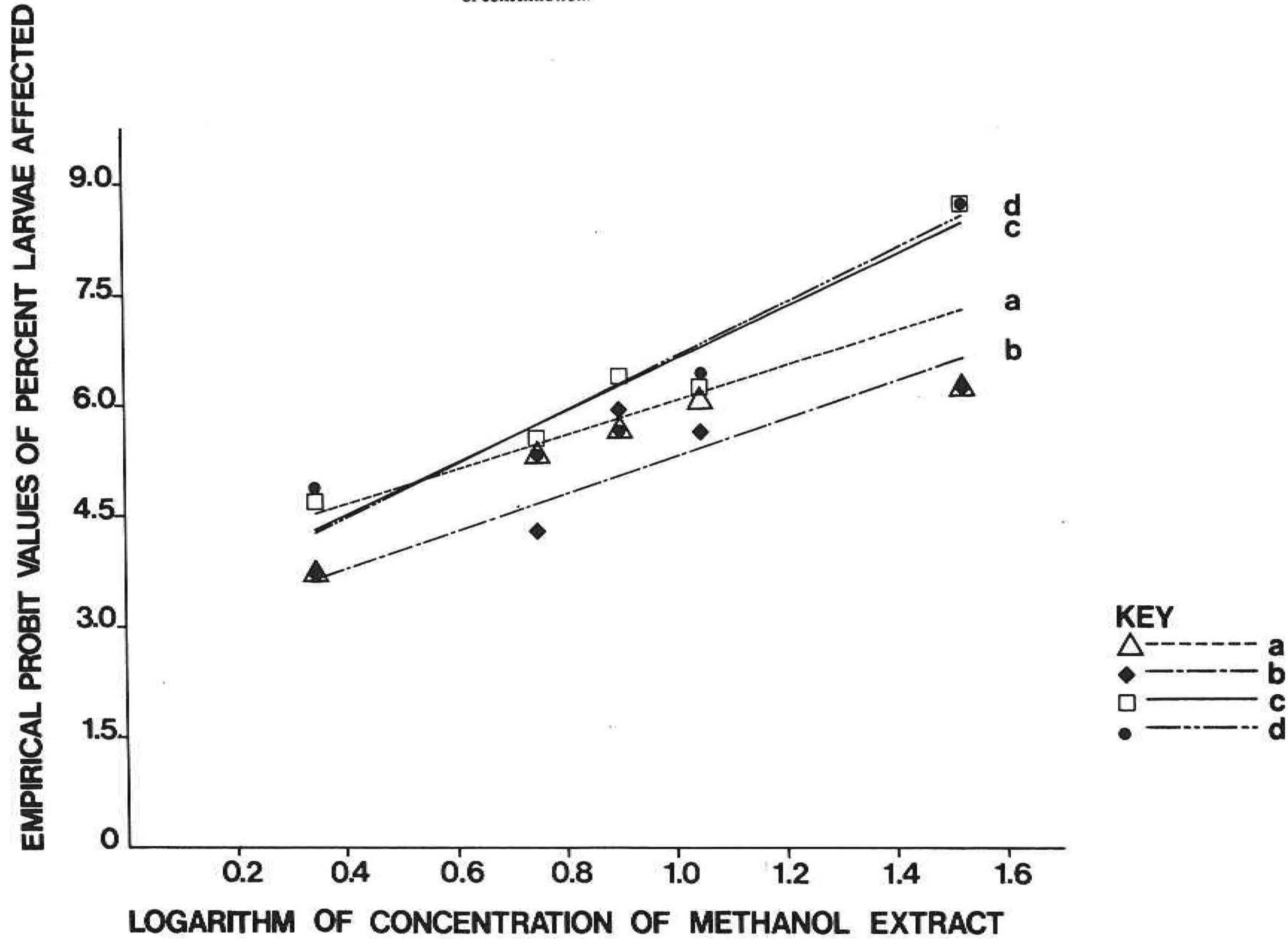


Table 1. Mortality effect of methanol extract on 3rd instar larvae of *A. aegypti*. Two batches of larvae were used one for bioassay A and B and the second C and D

		EXPERIMENT							
Dose No.	Concentration in mg. ml.$^{-1}$	a		b		c		d	
		Number Tested	Number Affected	Number Tested	Number Affected	Number Tested	Number Affected	Number Tested	Number Affected
1	3.354	20	18	20	18	20	20	20	20
2	1.118	20	17	20	15	20	18	26	24
3	0.783	20	15	20	11	20	15	24	18
4	0.559	20	13	20	5	24	17	30	19
5	0.224	20	3	20	2	29	12	39	17
Control	0.000	20	0	20	0	29	0	39	0

Table 2. The LD_{50} and LD_{90} Values of Methanol Extract against third instar larvae of *A. aegypti*

	Correlation Coefficient	50% Lethal Dose in mg ml–^{1}	90% Lethal Dose in mg ml.–^{1}
Experiment			
a	0.8424*	0.469	2.150
b	0.9380***	0.798	2.866
c	0.9132**	0.361	0.869
d	0.9057**	0.360	0.867
***P<0.01			
**P<0.02			
*P<0.05			

The effect of infection by *Trypanosoma brucei brucei* on the activity of bovine trypsin and/or proteases in the midgut of *Glossina morsitans morsitans*

R. M. W. Vundla, L. H. Otieno, D. L. Whitehead and E. Mpanga

Leucine aminopeptidase (AP); (EC 3.4.1.2.) and trypsin/proteinase VI (TP; EC 3.4.4.4.) activity in the midgut of infected and uninfected flies were monitored. During the 24 hours which elapsed after the teneral flies were fed on infected rats, the TP activity was lowered considerably by the presence of trypanosomes whereas AP activity was unaffected.

Contrary to the expectation which these results raised, added bovine trypsin (BTP) was not inhibited by the presence of trypanosomes in the gut but the opposite occurred; namely, a significant increase in the ability to hydrolyse synthetic substrate was manifest.

Materials and methods

The *T. b. brucei* used was EATRO strain 1969 and the *G. m. morsitans* originated from pupae obtained from the Tsetse Research Laboratory, University of Bristol, U.K. Teneral female flies were fed on a Wistar rat infected with *T.b. brucei* at the first peak of parasitemia. Control flies were fed on uninfected rats.

Dissection of the posterior midgut of the flies was performed at 4, 24 and 48 hours after feeding. 5–10 midguts were disssected and homogenised in chilled (4°C) 2×10^{-1}M phosphate-buffered saline glucose (PSG), pH 8.0 before centrifugation at 2,000 g for 20 min. to remove cell debris. For the studies using commercial bovine trypsin, midgut homogenates were prepared in a similar way.

The enzyme assays were performed using a Perkin-Elmer 402 UV Spectrophotometer. AP was assayed by the method of Wachsmuth (1966) under the following conditions: the substrate was 2×10^{-3}ML-leucine-p-nitroanilide (LpNA) with 10% dimethyl formamide (DMF)in 5×10^{-2}M phosphate buffer, pH 8.0 ($\Delta\varepsilon_{410}$= 8,800).

TP was assayed by the method of Erlanger (1961) using 5×10^{-2}M N–benzoyl-DL–arginine–p–nitroanilide hydrochloride (BApNA) with 6.6% DMF ($\Delta\varepsilon_{410}$= 8,800). When bovine trypsin (Merck, Darmstadt) was added to a final concentration of 173μg ml^{-1}, the mixture was preincubated for 30 min. before addition of BApNA. For this test, guts exposed to infection for 24 hours were used.

Results

Table 3 shows the results obtained for AP and TP at 4, 24 and 48 hours after feeding. There was no difference in the AP activity but the TP activity showed a decrease of 19% and 15% at 3, 24 hours respectively. There was no decrease 48 hours after feeding.

The results of the second part of the experiment showed no significant difference between infected and uninfected guts (Table 4). When BTP was added to gut homogenate there was a significant increase (P<0.001) in the ability to hydrolyse BApNA when compared with the results obtained for commercial trypsin alone (Table 4).

Table 3. The effect of Trypanosome infection on tsetse gut trypsin and aminopeptidase activity

UNITS		A P ACTIVITY µ moles/min/gut			T P ACTIVITY µ moles/min/gut		
Time (hours)		4	24	48	4	24	48
Infected	x̄	0.092	0.121	0.187	0.322	0.400	0.359±0.1
	S.D	±0.050	±0.070	±0.170	±0.091	±0.204	±0.125
Control	x̄	0.081	0.196	0.196	0.397	0.472	0.367
	S.D	±0.040	±0.080	±0.110	±0.119	±0.163	±0.079
	n	11	12	8	12	13	8
"t" test		NS	NS	NS	p=<0.2 *	p=<0.4	NS

	Source	Sum of SQ.	D.F.	Mean S.Q	F	P
Analysis of Variance for TP activity at 4 & 24 hours	Treatment	0.120314	1	0.120314	3.88	0.075
	Time	0.122550	1	0.122550	3.95	<.075
	Interaction	0.000045	1	0.000045	<1	
	Residual	1.426810	46	0.031018	—	
	Treatment	0.120314	1	0.120314	3.96	<0.075
	Time	0.122550	1	0.122550	4.03	<.05
	Residual	1.426855	47	0.030360	—	

*Wilcoxon's 2 sample test (non parametric) shows a significant difference (P = <0.04.) between infected and control for TP at 4 hours

Table 4. The effect of commercial bovine trypsin on tsetse gut trypsin/proteinase vi activity

		BApNA hydrolysis (ΔOD/Min)		
		Infected gut—Infected with BTP Gut	Control—Control with BTP	"t" test
	x̄	0.060	0.064	
	S.D	±0.007	±0.011	not significant
	n	8	8	
Bovine trypsin alone	x̄	0.040	0.040	
	S.D	±0.004	±0.004	
	n	7	7	
"t" test		p<0.001	p<0.001	

Discussion

The decrease in the trypsin/proteinase VI activity of infected guts indicates interference by the trypanosomes. This could be a direct effect (i.e. the trypanosomes may secrete a trypsin inhibitor) or an indirect one caused by the inhibition of trypsin/proteinase VI synthesis and/or secretion. Previous work (Annual Report, 1978) showed that incubating bloodform trypanosomes with gut homogenates *in vitro* for up to 3 hours resulted in no change in the trypsin activity, suggesting that the effect is not a direct one. However, the inability to detect any changes could have been due to the short time of incubation and the fact that the incubation was done at 4°C.

Because the trypsin proteinase VI secreted by the midgut cells of the tsetse was partially inactivated by the presence of trypanosomes, it was hoped that a trypsin inhibitor could be demonstrated. However, commercial bovine trypsin is not inhibited when incubated with the contents of infected guts—on the contrary, the bovine trypsin or the medium is activating the tsetse proteinases. The reason for this effect is being investigated. Nevertheless, the trypanosomes must inhibit the tsetse gut trypsin (but for some reason not the commercial enzyme) because the expected increase due to the endogeneous trypanosome proteases is not observed, indicating that the decrease is much greater than it appears to be.

Identification of ecdysone and ecdysterone in the eggs of the tick *Rhipicephalus appendiculatus*

David Whitehead

The eggs were washed and sedimented from ethanol then homogenised in 4x volume of ethanol before the debris was removed by centrifugation. The alcoholic extract contained ecdysteroids (1—3µg ml^{-1} eggs) which compete in the radio immunoassay (R I A) with ^{3}H-ecdysone (gift from Prof. P. Karlson) for the H21B anti-serum of Prof. J. D. O'Connor. The extract was concentrated, diluted with water then extracted with butan-1-ol saturated with water. The butanol layer, after further washes with water, was dried before elution with chloroform. The lipids in the chloroform eluted through a column of silica gel whereas the polar steroids were absorbed until the 1:4 ethanol: chloroform eluate from the dried butanol residue was in its turn passed down the same column.

The *Musca* bioassay indicated that 11.56 ng ml^{-1}

eggs of moulting hormone was eluted in chloroform whereas 9.25 ng was present in the ethanol: chloroform mixture. The R I A already mentioned detected as much as 28.8 and 661 ng ml^{-1} in the two eluates respectively.

Chromatography of the ethanol: chloroform eluate on silica gel (TLC), followed by R I A of the eluate from a band migrating alongside authentic hormone showed that ecdysterone (35.4ng ml^{-1}) and ecdysone (75 ng ml^{-1}) were present. The bioassay showed again less ecdysteroids than the R I A (i.e. 0.78 and 0.61 ng ml^{-1} of ecdysterone and ecdysone respectively) in the eluates. As much as 50% could have been lost during chromatography on TLC. Thus in future HPLC and subsequently GC with ECD will be used to measure the ecdysteroids in tick eggs and haemolymph. A more complete analysis of eggs is necessary to ascertain whether the steroids are bound to proteins or whether they occur free or conjugated as sulphate or glucuronide.

Attempts at Muguga to immunize rabbits against tick nymphs have been made with the extracts of whole eggs. The partial success which was achieved will now be followed by innoculation of both rabbits and cattle with the steroid-rich component among others obtained after fractionation of lipoproteins and proteins in the eggs.

(Collaborators—F. Obenchain, C. Mango)

Director of Research
Professor T. R. Odhiambo

Research Adviser
Professor M. Locke (1977)

Research Staff
Mr. M. Chimtawi (1974) Principal Technician
Dr. E. D. Kokwaro (1975) Research Scientist
Mrs. J. A. Kongoro (1974) Research Assistant
Mr. P. Lisamula (1973) Senior Technician
Mr. J. Owor (1973) Associate Scientific Officer
Mrs. J. Murithi (1979) Senior Technician
Mr. N. Ogoma (1979) Junior Technician

Histology and Fine Structure research unit

The Unit has provided support services to a number of ICIPE research programmes. Particular emphasis has been placed on ticks, mosquitoes, tsetse flies, rice planthoppers and legume pod-borers.

Preliminary fine structural investigations on sensory structures found on various tick species are underway. This work is done in collaboration with the Sensory Physiology Research Unit.

The SEM has helped distinguish the fine structural differences in the pattern and arrangement of sensillae (Fig. 1) on the rice brown planthopper, *Nilaparvata lugens* (Ståhl) biotypes. This material was sent from the International Rice Research Institute (IRRI) which is collaborating with ICIPE'S Bases of Plant Resistance Programme. In another study conceived to clarify the larval instars of *Maruca differentialis*, the SEM has revealed headcapsule structures hitherto useful in the classification of larval instars. Identification of species

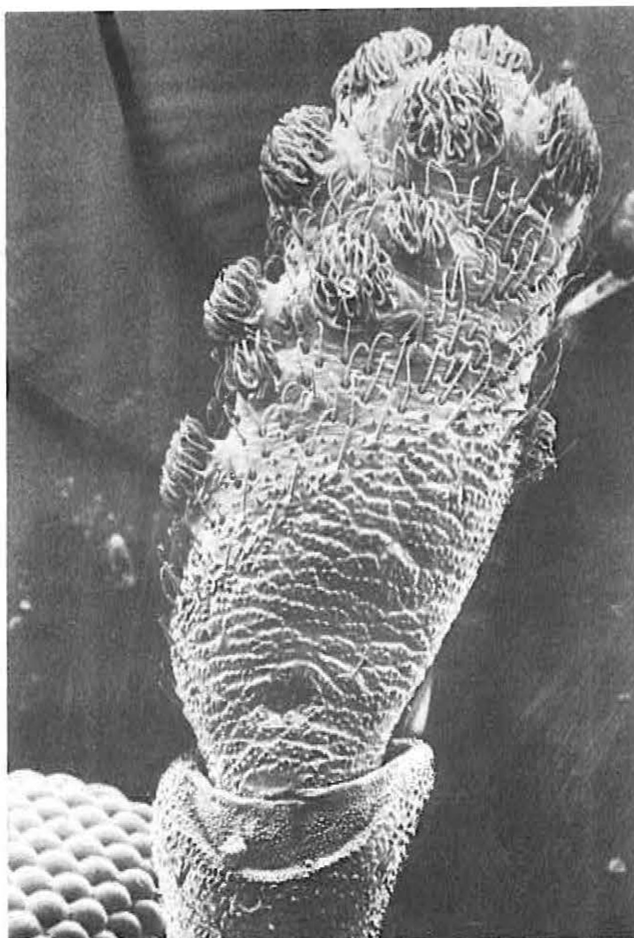


Figure 1. SEM of sensillae (S) on antenna of the brown planthopper *Nilaparvata lugens*. × 900.

of a fungus (*Coelomomyces* sp.) obtained from the Kenya Coast and showing high pathogenicity to malaria carrying mosquito (*Anopheles gambiae*) has shown observable morphological structures. This work is done in collaboration with the Medical Vectors Programme.

Electron microscopy has been employed to follow the incorporation of horseradish peroxidase into the milk gland of *G. morsitans*. Within four hours, activity is present and can be detected bound to parts of cell membranes and within the cytoplasm. This study complements the research going on in the Tsetse Reproductive Physiology Programme.

The Unit has also collaborated with a number of other institutions, especially within Africa as contained in the following section.

Eggs of *Bathycoelia thalassina* (H–S) a pentatomid pest of cocoa pods, sent to us from the University of Ibadan, West Africa were examined with the SEM. The nature of the existing structures, such as the micropylar processes situated at the neck region, the chorion and the operculum are shown in Figure 2.

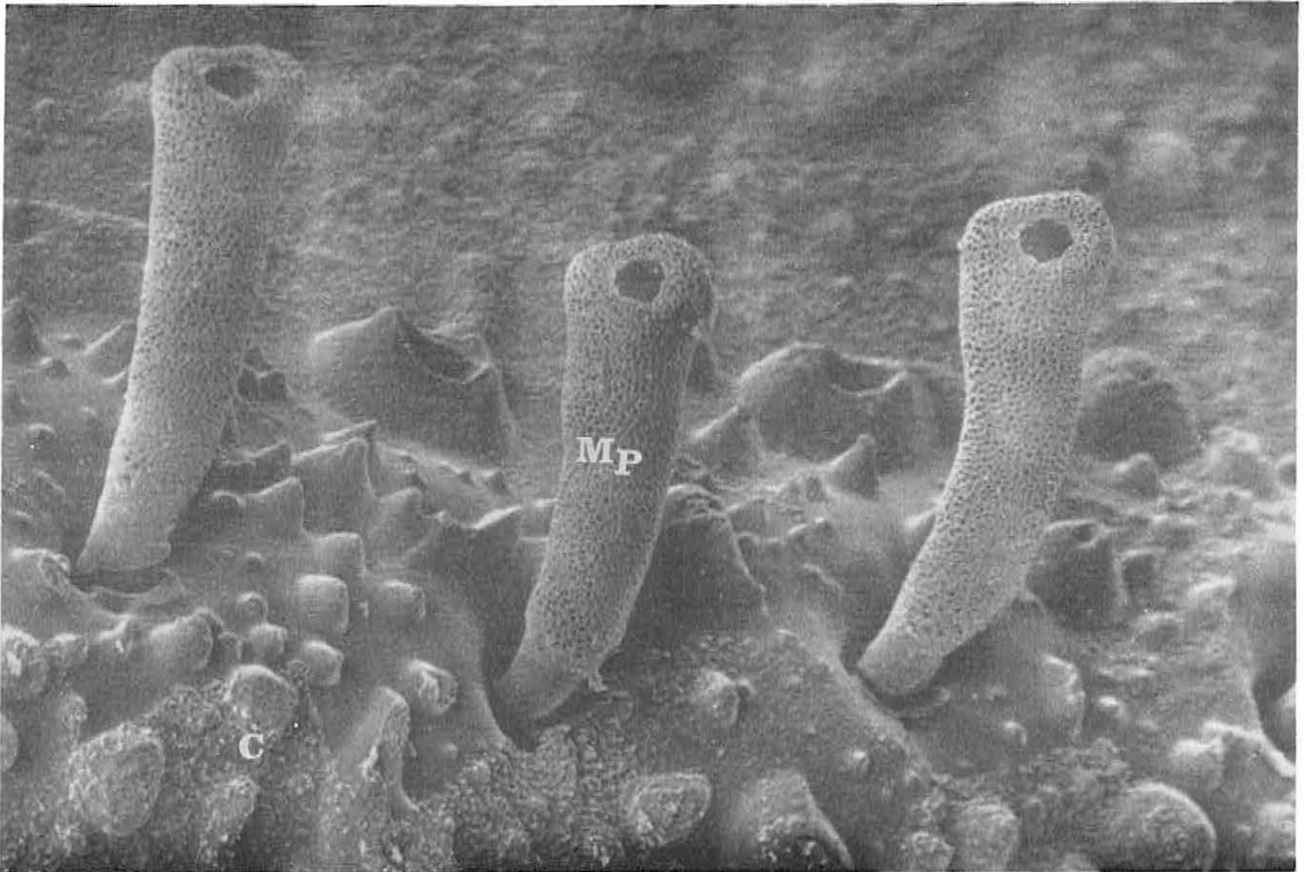


Figure 2. SEM of neck region of egg of *Bathycoelia thalassina* showing micropylar processes (Mp) and part of the chorion (C). ×30,000.

A comparative scanning electron microscope study of three maize varieties sent to us from the University of Ife, West Africa was carried out. These varieties of maize appear to show varying degrees of susceptibility to weevil damage.

Collaborative work with the Department of Crop Science, Nairobi University, have centred on the identification and characterization studies of some viruses (Fig. 3) affecting legumes and vegetable crops in Kenya. Similarly, ultrastructural studies of the salivary glands from infected ticks and the placental barrier in the cane rat and elephant shrews were continued in collaboration with the staff of the Department of Veterinary Anatomy, Nairobi University. In addition, morphological studies of the male accessory reproductive glands of *Dysdercus fasciatus* were continued in collaboration with the Department of Zoology, Nairobi University.

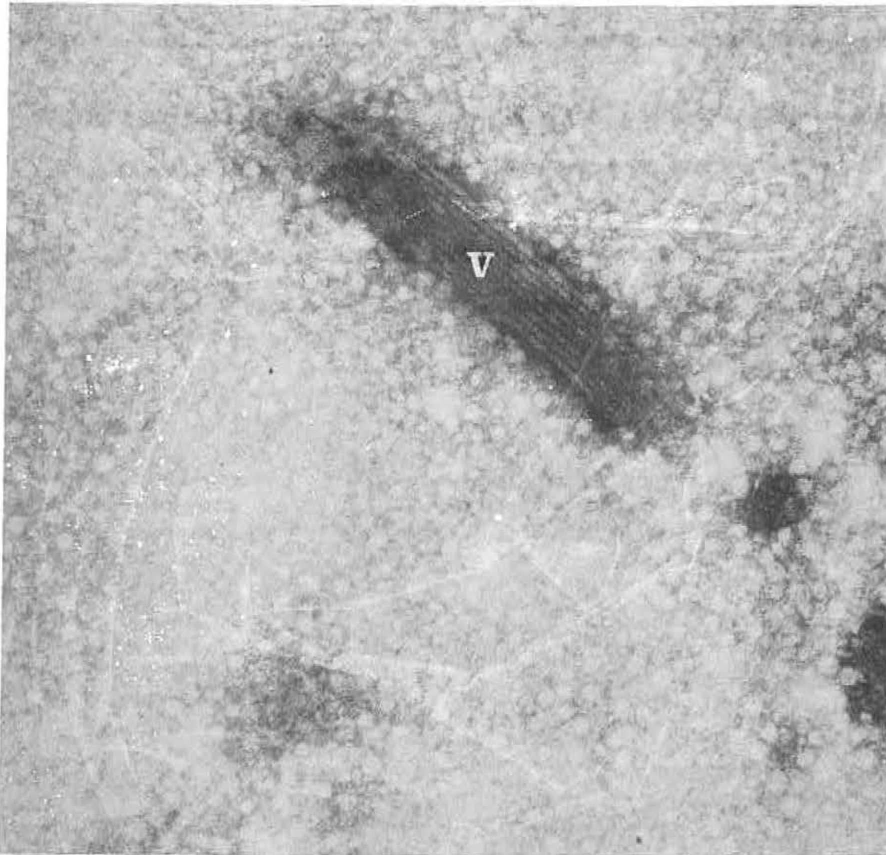


Figure 3. A group of viruses (V) isolated from beans (*Phaseolus vulgaris* L.). · 101,250.

Spermatophore of the Tsetse Fly *Glossina Morsitans*

E. D. Kokwaro

Sperm sacs or spermatophores occur in a wide variety of insect species which employ internal fertilization. Spermatophores are probably formed from secretory products of the accessory glands. The ultrastructure of the spermatophore body wall in *Glossina morsitans* was examined to find out whether it had any ultrastructural details in common with the male accessory gland secretions.

The spermatophore of *G. morsitans* consists of a central mass of sperm surrounded by a body wall (Fig. 4). At the electron microscopic level, the body wall contains filaments (Fig. 5), matrix (Fig. 5), dense aggregates of material (Fig. 5), a felt of microfibrous components (Fig. 6) and granules (Fig. 5).

Similarities exist between the male accessory gland secretions of *G. morsitans* and those of the spermatophore body in so far as the spermatophore body wall contains filaments, dense aggregates of material, matrix and clusters of granules. The matrix has an ultrastructural appearance similar to that of the homogeneous secretion found in the proximal part of the male accessory reproductive glands. As shown in the sixth ICIPE annual report (1978) these features are common in the male accessory gland secretions. The filaments, clusters of granules and dense aggregates of material are distributed throughout the spermatophore body wall. This is also characteristic of most of the accessory reproductive glands secretion except in the proximal

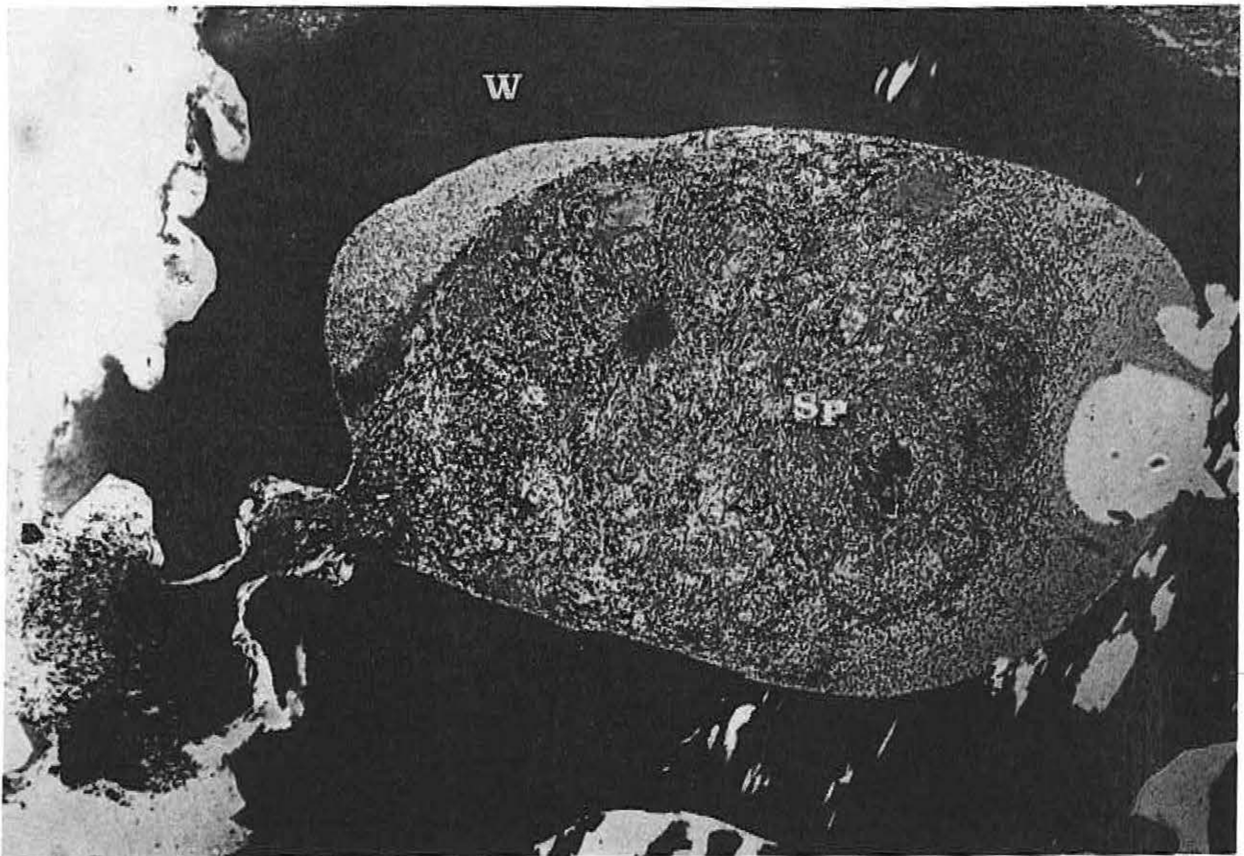


Figure 4. Photomicrograph of the spermatophore body wall (W) and a central sperm mass (Sp). 138.

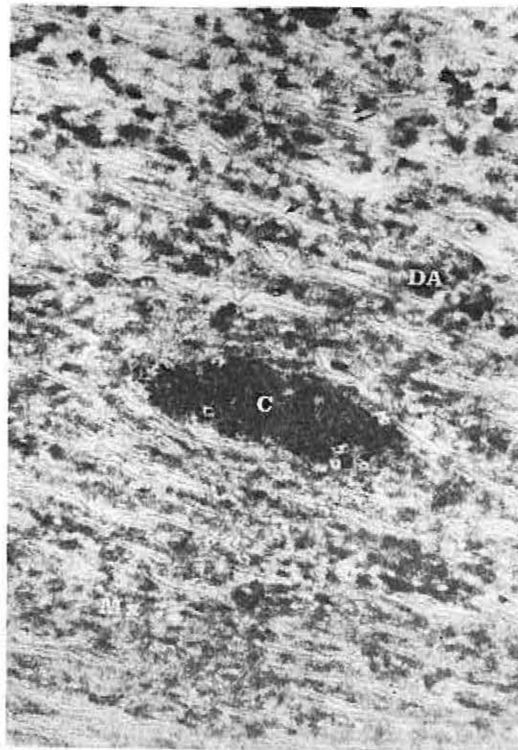


Figure 5. TEM of the spermatophore body wall showing filaments (arrows), clusters of granules (C), matrix (Mx) and dense aggregates of material (DA). × 97,500.

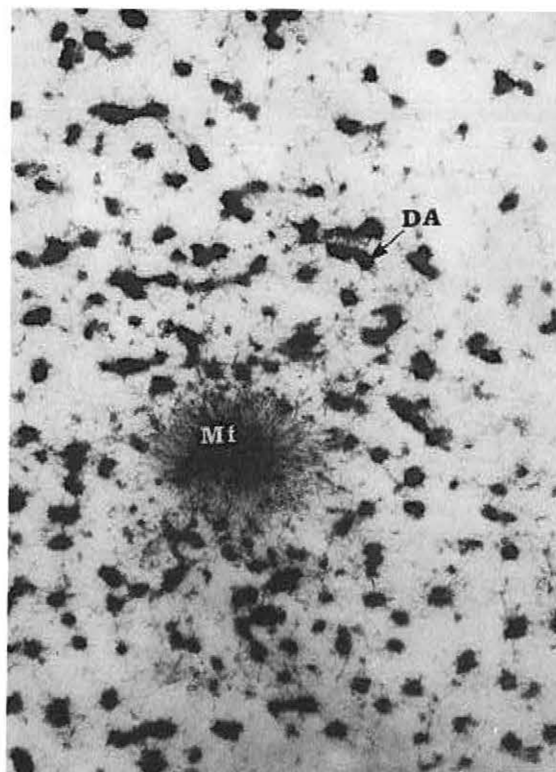


Fig. 6. TEM of the spermatophore body wall showing the microfibrous component (Mf). Dense aggregates of material (DA). × 97,500.

part where only a homogeneous mass is found in the lumen. Accessory secretions of male insects have been shown to have a variety of functions aside from forming components of the spermatophore, including such things as sperm and muscle activation. Present observations on *G. morsitans* spermatophore body wall have confirmed that the sperms are surrounded with material which is ultrastructurally similar to the secretions of the male accessory glands.

Ultrastructural changes in the glandular epithelium of the tsetse fly, *Glossina morsitans* Uterus

J. A. Kongoro

Following the establishment of the basic ultrastructure of cells of the ventral glandular epithelium of the uterus of *G. morsitans* (ICIPE Annual Report 1978), further work is being conducted to elucidate ultrastructural changes in these cells.

The methods used have been described in ICIPE Annual Report, 1978. Preliminary results indicated that glandular cells (Fig. 7) in seven-day old mated flies had more autophagic vacuoles (AV), than cells in teneral flies. Ribosomes were organised into rough endoplasmic reticulum (RER), Fig. 8. In cells of young flies, free ribosomes were abundant and the rough endoplasmic reticulum was not well developed. Progressive protrusions of the apical surface of the cells indicated exocytotic activity (Ex) in the cells (Fig. 9). From these observations it is deduced that cells at this stage are probably synthesizing a substance which is released into the lumen by exocytosis.

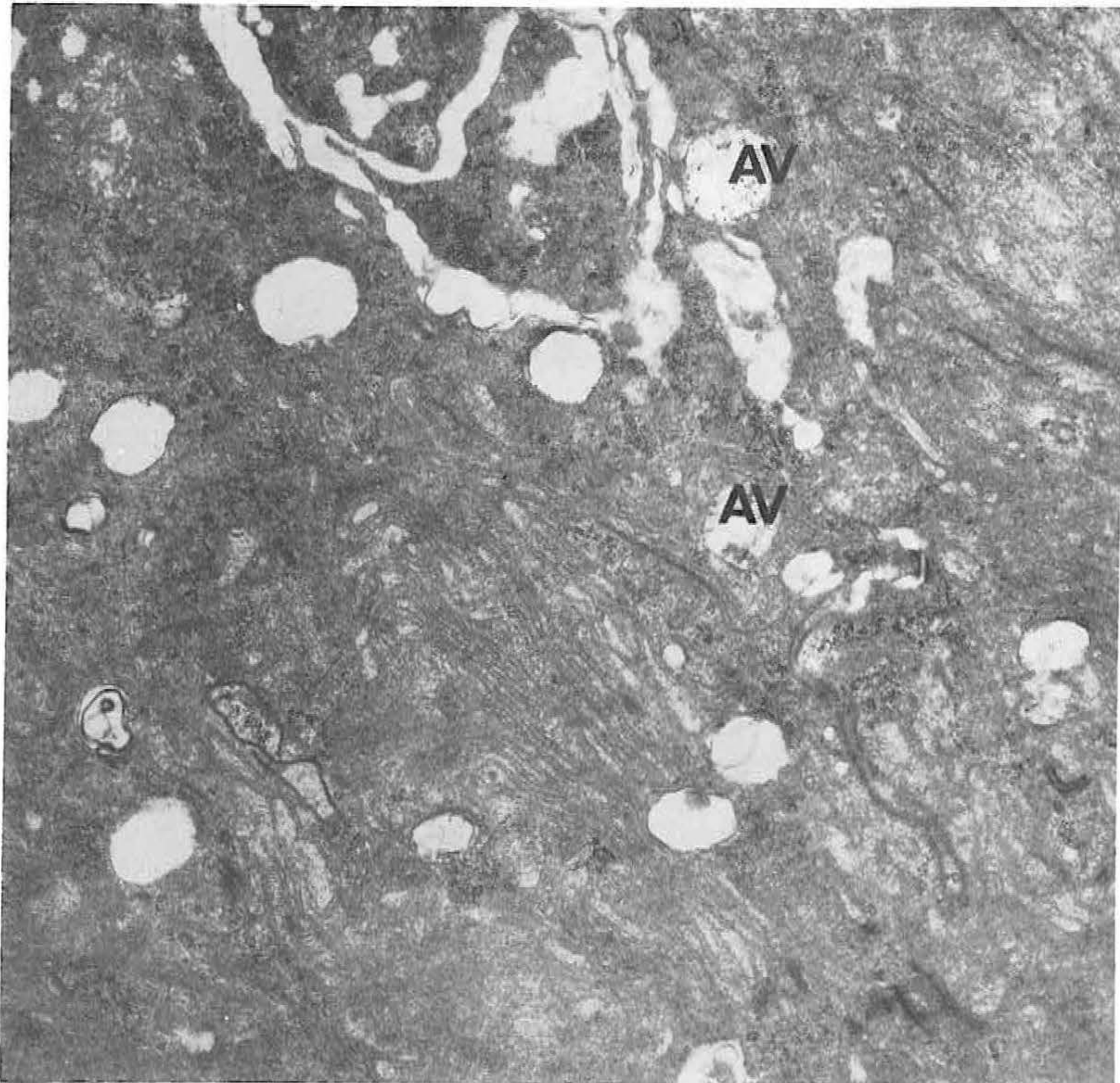


Figure 7. A low magnification field covering a part of the glandular epithelium of the uterus of a seven-day old mated *G. morsitans* AV: Antophagic vacuoles (× 20,000).

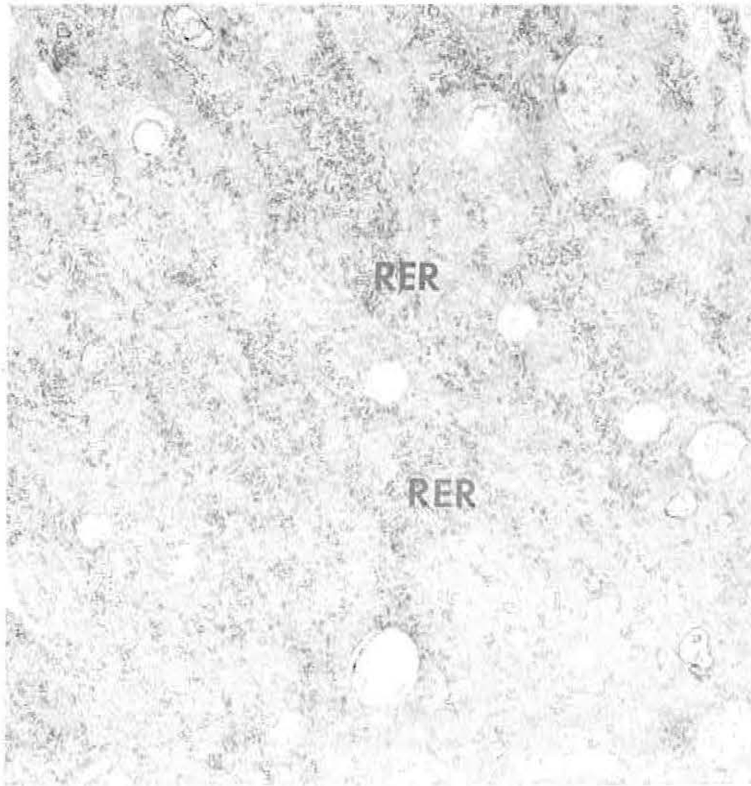


Figure 8: A part of a cell from the glandular epithelium of *G. morsitans* uterus showing the rough endoplasmic reticulum RER (× 20,000).

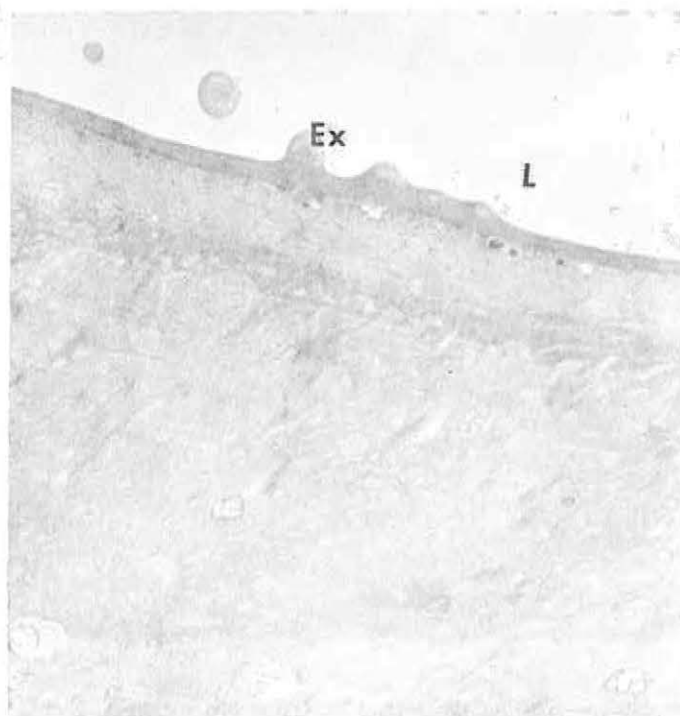


Figure 9. A low magnification field showing the apical part of a cell from the glandular epithelium of the uterus of *G. morsitans* Ex. Exocytosis L: Lumen (× 20,000).

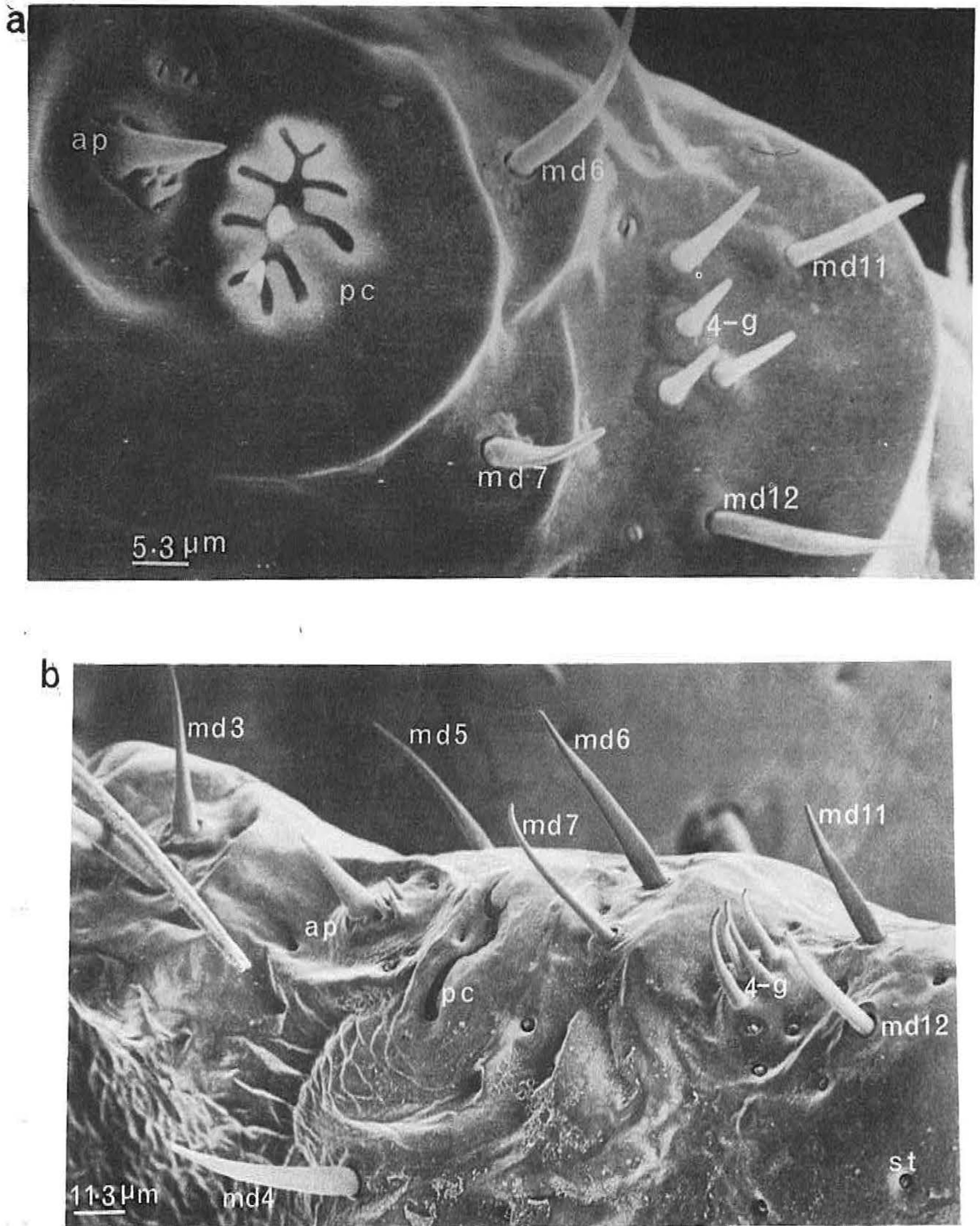


Figure 10. Scanning electron micrographs showing the medial dorsal sensilla on tarsus I of

(*a*) *Rhipicephalus appendiculatus*

(*b*) *Amblyomma variegatum*

Note: Medial dorsal sensilla md 2-7 and 11-12 four group sensilla (4-g), Halleris organ composed of anterior pit sensilla (ap) and posterior capsule (pc); campaniform sensilla (st)

Identification of a thermal receptor sensilla on tarsus I of the Ixodid tick *Rhipicephalus appendiculatus*

S. M. Waladde, E. D., Kokwaro and M. Chimtawi

Scanning electron microscopy has revealed that the sensilla pattern on the dorsal surface of tarsus I is similar among vàrious tick species; but the sizes and orientation of those sensilla differ (Fig. 1). Apparently very little is known about the specific functions of most of those sensilla. Because of this gap in our knowledge, the work in progress endeavours to bridge this gap by using ultrastructural, electrophysiological and behavioural methods. The same methods are being applied on other tick sensilla which are equally intriguing.

Electrophysiological tests on *Rhipicephalus appendiculatus* have shown that sensillum md 7, located posterior to the Hallers organ (Fig. 10a), has a spontaneous activity which is altered by sudden changes in the ambient temperature. Action potential from the neurone/s in md 7 were picked up by a recording tungsten electrode introduced into the base of the sensillum while the indifferent electrode was in the abdomen. The signals were amplified and recorded in the usual manner. Changes in the ambient temperature were brought about by a puff of warmed or chilled air directed towards the sensillum. Warm air was drawn from an air chamber heated with hot liquid paraffin while cold air was drawn from a chamber either cooled with iced water or dry ice. Air that was cooled with dry ice lowered the ambient temperature by more than 5°C and this caused a ten fold increase in the impulse frequency (Fig. 11). Application of warm air inhibited the spontaneous activity and this was followed by a rebound as soon as the warm air current was turned off. The above two effects caused by warm air were also observed when the fiber optic lamp, illuminating the tick preparation, was switched on and off. The effect of this lamp was proportional to the intensity of its light (Fig. 12). Although the fiber optic lamp is supposed to produce cold light, thermocouple tests showed that the lamp generated some heat which caused the observed changes in the spontaneous activity from the md 7 sensillum. These kind of responses to rapid temperature changes have been observed among other arthropods and it will be interesting to discover the distribution of temperature receptors on tick tarsi and other parts of the body.

Transmission electron microscopy has shown that md 7 is a thick walled sensillum with more than one opening at its tip. Its distal end is marked by two lumina which merge proximally. One of the lumina contains Six dendrites bathed by a granular sensillum liquor. The second lumen has a luminal fluid which is more electrontranslucent than the sensillum liquor (Fig. 13a). It is interesting to note that those ultrastructural details are common to some contact chemoreceptors. It is therefore not good practice to determine sensilla function on the basis of ultrastructural observations alone. However the structural details of md 7 are entirely different from those of the neighbouring four-group (4–g) sensilla (Figs. 10 and 10b). We are interested in knowing whether the function of md 7 is uniform among other tick species.

As shown in Fig. 7 the arrangement of sensilla on tarsus I provides an open invitation for further studies and the outcome of this work is expected to provide information on what stimulates the ticks and the medium through which ticks perceive stimuli.

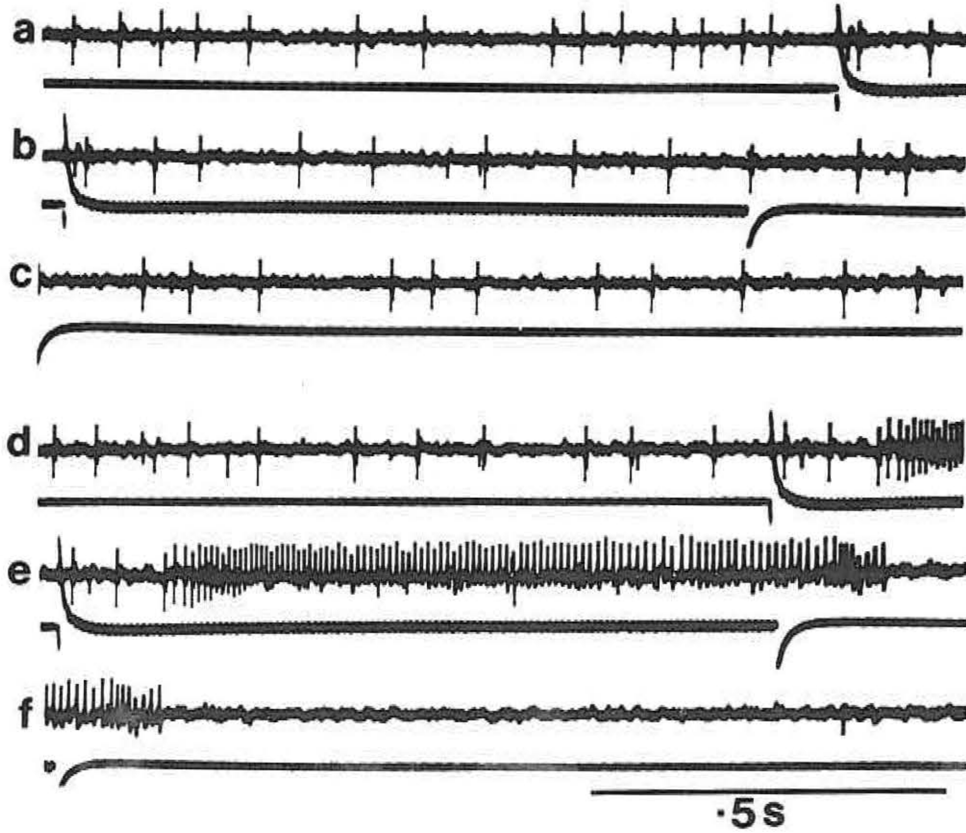


Figure 11. Single cell responses from sensillum md 7 to ambient temperature and to a current of air chilled by dry ice. The wavy line below a trace indicates duration of stimulus application.

a–c Continuous overlapping record showing that switching on and off of the air delivery system did not alter the spontaneous activity; (a) prestimulus, (b) dummy stimulus run (c) post stimulus

d–f Continuous overlapping record showing the effect of cold air stimulus. Delay from stimulus onset to changes in the spikepattern was due to artifact in the air delivery system.

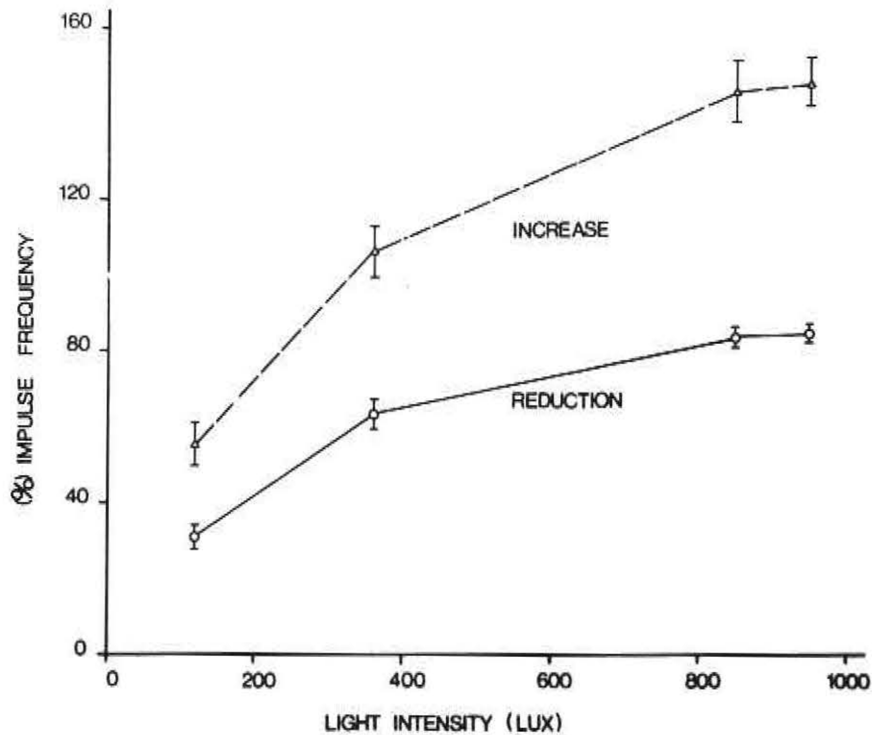
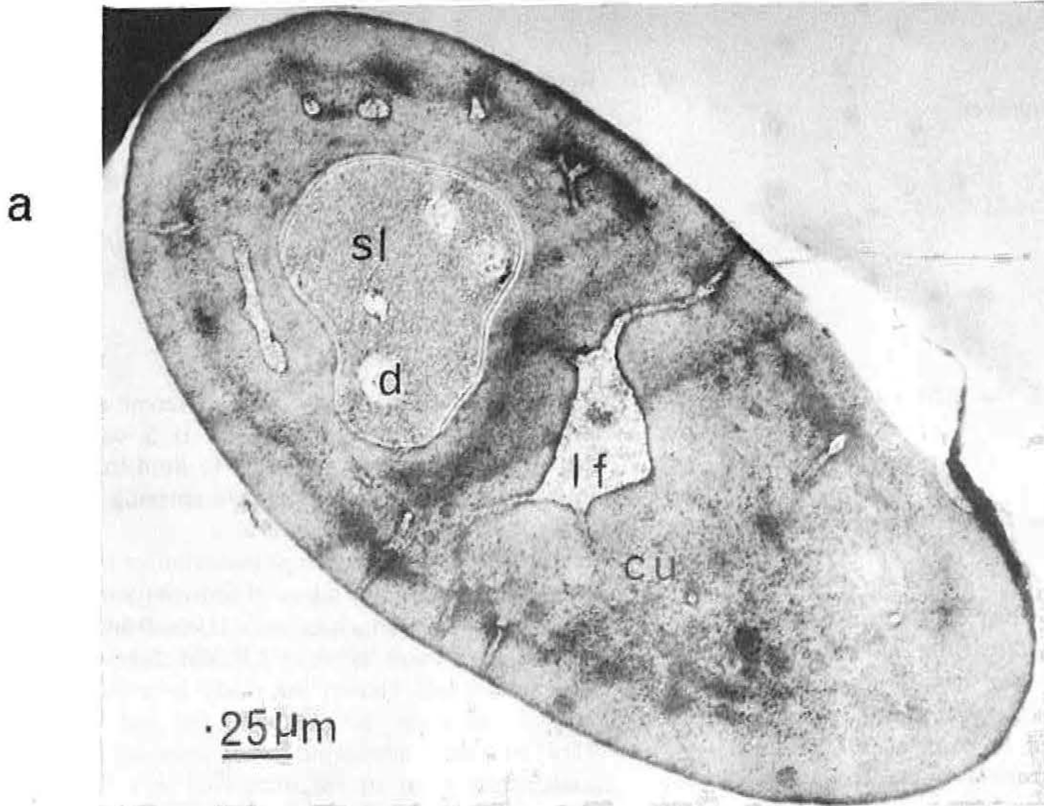


Figure 12. Graph showing the effect of light intensity on the spontaneous impulse activity from md 7 sensillum; light reduced the level of spontaneous activity whereas switching off the light caused immediate rebound with increased frequency; ± SE of % impulse frequency.

a



b

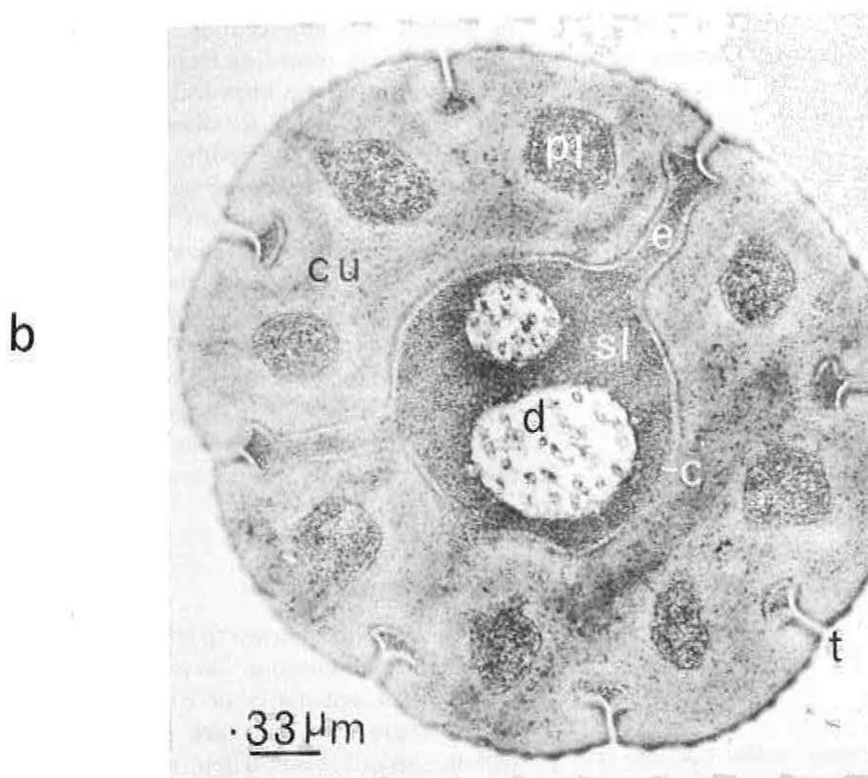


Figure 13. Transmission electron micrographs showing differences between transerse sections cut through md 7 sensillum (13a) and one of the neighbouring four group sensillum (13b): Cuticle (Cu) sensillum liquor (sl) dendrite (d) luminal fluid (lf). Channels to the exterior (e) external slit (t) peripheral lumina (pl) central lumen (c).

SENSORY PHYSIOLOGY RESEARCH UNIT

Research Advisor
Professor L. M. Schoonhoven

Research Staff
Dr. J. V. Clark (1976) Postdoctoral Research Fellow
Mr. H. M. Kahoro (1975) Technician
Dr. H. MacFarlane (1977) Research Scientist
Mr. R. K. Saini (1976) Scientific Officer
Dr. S. Waladde (1978) Posdoctoral Research Fellow

Gustatory responses of the African armyworm

J. V. Clark

1. Feeding rate of the last instar related to receptor sensitivity

The feeding rate of last instar larvae on maize leaves has been studied, using larvae obtained from the ICIPE insectary. Late fifth instar larvae were placed singly in 1lb Kilner jars with a piece of fresh maize leaf. The jars were placed in an oven at 30°, 50–70% RH. At approximately eight hour intervals each larva was weighed and the area of the maize leaf eaten was traced onto a piece of paper and later estimated gravimetrically. The mean weights and feeding rates for thirteen larvae are shown in Fig. 1. It may be seen that feeding falls off very abruptly at about sixty hours from the last larval moult, and this cessation of feeding is accompanied by a very rapid weight loss. This weight loss cannot be attributed to the emptying of the gut, as faecal pellet weighings show that they represent only a small proportion of the fall in weight. It is suggested that the weight loss is due primarily to fluid loss caused by epidermal gland secretion prior to entering the prepupal phase.

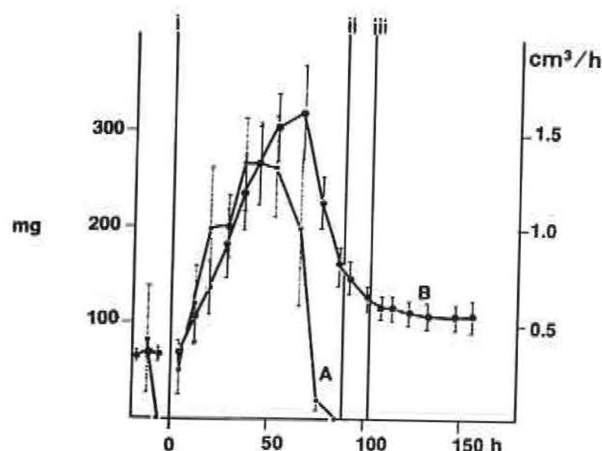


Figure 1. Weight changes (B) and mean feeding rate over the preceding interval (A) averaged for thirteen larvae. Weight in mg, feeding rate in cm²/h. Vertical bars represent twice standard error. Abscissa: estimated time from moulting from fifth instar. Lines (i), (ii), (iii) represent mean times of moulting, prepupation and pupation.

To determine if the abrupt cessation of feeding in the last instar is caused by a loss of sensitivity of the mouth-part receptors, recordings were taken from the mouth-parts of sixth instar larvae at different stages in the instar. The mouthparts chosen for study were the previously studied styloconic sensilla, and the test stimuli used were sucrose, adenosine and meso-inositol, all at concentrations of 10^{-2}M, dissolved in a 10^{-1}M NaCl electrolyte. Caterpillars for recording were divided into four groups, on the basis of the earlier feeding studies: These were—1: 100–200mg, increasing in weight. 2: 200mg and above, increasing in weight. 3: decreasing in weight (and non-feeding). 4: prepupal larvae.

Standard tip recording techniques were used, and the responses to the test chemicals were scored for 100msec after a 20msec delay to allow for the contact artefact of the electrode. The results of these experiments are shown in Fig. 2. It can be seen that there is no sudden change in receptor sensitivity over the course of the last instar, at least for the chemicals tested. In fact the adenosine response (from the lateral sensillum styloconicum) increases, although the significance of this increase is not great ($r = 0.798$). Thus it seems from these results that the abrupt cessation of feeding in the last larval instar of *S. exempta* is not due to a loss of receptor sensitivity, and that central nervous system changes are likely to be involved in the termination of feeding.

Feeding deterrent studies

Two chemicals, known to stimulate deterrent receptors in other lepidopterous larvae, have been used in a study of the sensitivity of sixth instar larvae to deterrents. These chemicals are salicin, a glucoside, and caffein, an alkaloid. Their effects have been studied at the receptor level and at the level of the whole larva.

Electrophysiological recording from the labrum has shown that normally there is no response to these two chemicals, tested at a concentration of 10^{-2}M with a

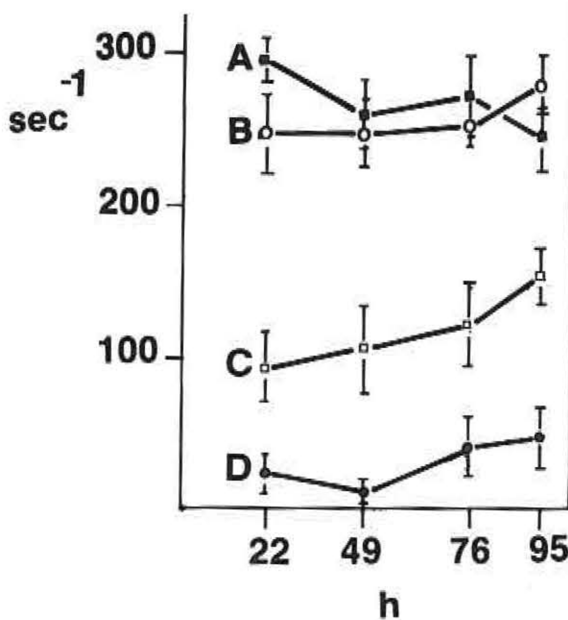


Figure 2. Receptor responses to test stimuli at various times over the last instar. Ordinate: mean impulse frequency (with twice standard error): About fifteen responses per mean. Abscissa: estimated mean time from moulting for each group used. A: medial meso-inositol receptor. B: lateral sucrose receptor. C.: lateral adenosine receptor. D: medial sucrose receptor. All stimuli at 10^{-2}M in 10^{-1}M NaCl electrolyte.

10^{-2}M NaCl electrolyte. However, there does seem to be an interaction between cells normally responding to sodium chloride and caffein, in that their spike heights are affected by caffein. This effect is not seen with salicin.

In the maxillary sensilla styloconica receptor responses to both salicin and caffein can be recorded (Table 1).

Table 1. Responses to salicin and caffein from the lateral and medial sensilla styloconica. Figures represent mean impulse frequency per second, with two times standard error. Number of preparations per mean also shown. Solutions made up in 10^{-2}M NaCl electrolyte

Stimulus	Caffein		Salicin	
Molarity	Lateral	medial	lateral	medial
10^{-2}M	4.5±11.4 (n=11)	12.3±9.2 (n=13)	11.6±5.8 (n=19)	43.9±17.2 (n=13)
10^{-3}M	25.6±7.6 (n=9)	9.1±7.8 (n=11)	1.3±1.8 (n=15)	15.4±12.4 (n=13)
10^{-4}M	2.9±4.4 (n=14)	3.3±5.2 (n=12)		
10^{-5}M	0.0 (n=10)	14.2±9.2 (n=11)		
10^{-2}M NaCl	0.8±1.6 (n=13)	14.2±9.2 (n=13)	0.0 (n=18)	14.7±13.4 (n=13)

The lateral sensillum styloconicum responds to caffein with spiking from a single neuron, which falls below threshold between 10^{-3}M and 10^{-4}M. The medial sensillum does not respond to caffein. The lateral sensillum styloconicum responds to salicin with two spike types, one of which seems to be from the caffein sensitive neuron. The medial sensillum reponds to salicin with two spike types. Both sensilla cease to respond below 10^{-2}M salicin. Recording with mixtures of sodium chloride and caffein, sodium chloride and salicin, suggest that in the lateral sensillum one of the cells responding to sodium chloride is caffein and salicin sensitive, whilst in the medial sensillum the two sodium chloride sensitive cells both respond to salicin. These results explain why sodium chloride is a very effective deterrent of feeding activity of larvae on sucrose treated discs. Caffein seems to have effects other than stimulation of one cell in the lateral sensillum styloconicum. Thus a mixture of salicin and caffein (both at 10^{-2}M) applied to the lateral sensillum results in a single spike, characteristic of the response to caffein alone. Preliminary experiments suggest that caffein does not, however, have any effect on sucrose receptors in the sensilla styloconica.

To establish that salicin and caffein do in fact act as feeding deterrents in the armyworm their effect on larvae feeding on sucrose treated glass fibre filter paper discs was measured. The larvae were presented with the treated discs for a period of 24 hours and the amount of feeding was determined gravimetrically.

Furthermore, to establish that the sensitivity to these two compounds resides in the maxillae, as suggested by electrophysiology, a number of feeding tests were run with larvae whose mouthparts had been ablated using a microcautery device or removed using iridectomy scissors.

It was found that both caffein and salicin acted as feeding inhibitors at high concentrations (see Table 2) though at lower concentrations they had no significant deterrent effect. Despite the fact that the electrophysiological results suggest that the sensitivity to salicin is less than that to caffein, and the fact that the feeding levels tend to support this finding, there are no significant differences between the feeding levels with salicin and caffein at similar concentrations (see Table 2).

Ablation experiments revealed no difference in feeding levels between maxillary cauterised larvae and controls, suggesting that receptors to these two deterrents exist apart from those demonstrated in the sensilla styloconica. This conclusion was confirmed by the last experiments in Table 2. Caterpillars had their maxillae cauterised, their labial palpi cauterised, and their labra removed with iridectomy scissors. One batch of larvae was presented with sucrose treated discs, and a second batch with discs treated with both sucrose and caffein. There was a significant inhibition of the feeding of the ablated larvae by caffein ($p < 0.01$), demonstrating the presence of other caffein receptors.

Table 2. Results of feeding bioassays. Means and number of larvae used shown, together with ANOVA (p) levels. See text for details

Disc Concentration	Unablated	(p) (ANOVA)	Maxillar Cautery
26,000ppm salicin 32,000ppm sucrose	1.3mg (n = 61)*	←N.S.+→	1.8mg (n = 28)+
2,600ppm salicin 32,000ppm sucrose	7.8mg (n = 27)	←N.S.→	10.2mg (n = 19)
18,000ppm caffein 32,000ppm sucrose	0.9mg (n = 46)+	←N.S.+→	0.9mg (n = 36)+
1,800ppm caffein 32,000ppm sucrose	3,4mg (n = 21)	←N.S.→	5.2mg (n = 21)
32,000ppm sucrose	7.3mg (n = 60)*		
	Unablated	**(p) (ANOVA)**	**Maxillar/Labial Cautery, Labrectomy**
32,000ppm sucrose 18,000ppm caffein	1.0mg (n = 51)+	→	0.0mg (n = 39)+
		<0.01 ← →	1.8mg (n = 41)+
		<0.01	
Distilled water	0.0mg (n = 57)+	←	

* Three assays.
+ Two assays

Comparison of the sucrose feeding level of ablated larvae with the level of feeding of unoperated larvae on discs treated with distilled water suggests the presence of previously unreported sucrose receptors.

The location of these caffein and sucrose receptors is as yet unknown, but it may be that they reside in the pharyngeal region.

Behavioural studies on *Macrotermes michaelseni* (Sjöstedt)

J. H. MacFarlane

Trail Laying Behaviour in *Macrotermes michaelseni:*
Major workers were allowed to establish foraging trails between a nest and foraging arena set-up in the laboratory. The workers were photographed with a cine camera at various time intervals to record the behaviour during trail laying. Some workers were allowed to walk over sooted slides to determine which part or parts of the termite body touched the substrate during trail laying.

The behaviour of major workers during trail laying can be divided into 3 major divisions which are further divided into 1 or 2 subdivisions. The behavioural types are as follows:

1a — Whole body of worker; head, thorax and abdomen is low and parallel to the substrate, centre area of abdomen lower than either the anterior or posterior end, heavy and numerous abdominal hair marks on the sooted slide.

1b — Same general description as 1a but have additional lowering of the sternal gland area of the abdomen, heavy and numerous abdominal hair marks on the sooted slide.

2a — Body of worker at a slight angle; head and thorax at a slight angle, head and thorax slightly raised from the substrate, posterior end of abdomen slightly lower than anterior end, 3/4 of abdomen low and close to substrate, light abdominal hair marks on the sooted slide.

2b — Same general description as 2a but have additional lowering of the sternal gland area of abdomen, light abdomen hair marks on the sooted slide.

3a — Body of worker at a large angle, head and thorax much higher than rest of body, anterior end of abdomen much higher than posterior half, 1/2 or less of abdomen low and close to substrate, light abdominal hair marks on sooted slide, lighter than those observed in 2a or 2b.

3b — Same general description as 3a except that abdomen is raised and lowered during movement, light and intermittent abdominal hair marks on the sooted slide.

3c — Same general description as 3a except that posterior end of abdomen raised clear of substrates, no abdominal hair marks on the sooted slide.

Behavioural types 1a and 1b were observed in the first workers leaving the nest when no previous trail had been established. It was assumed that these workers were laying a heavy trail. This behavioural was observed to continue for about 30 minutes. Workers leaving the arena were never observed in behavioural types 1a and 1b even within the first few minutes after the establishment of the nest.

Behavioural types 2a and 2b were observed in workers leaving the nest 30 minutes after the initial establishment of the trail and continued to be recorded for 24 hours when observations were stopped. Workers leaving the arena were observed in these behavioural types infrequently, 9% when leaving the arena to 58% when leaving the nest. It was assumed these workers were laying a lighter trail than either 1a or 1b since the trail had become established.

Behavioural types 3a, 3b and 3c occur most frequently in workers leaving the arena when the trail is being established and while workers from the nest are laying heavy trails as shown by behavioural types 1a and 1b. The workers in types 3a and 3b lay very light trails. They can be considered to be maintaining the trail rather than increasing its strength as in types 1a, 1b, 2a or 2b. Type 3c workers lay no trails. This was observed very frequently in workers leaving the arena. Workers leaving the nest showed behavioural types 3a or 3b only 1 hour after the initial establishment of the trail.

Longevity of natural trails from major workers of *Macrotermes michaelseni*

Major workers were allowed to lay trails on No. 11 typing paper on either arm of a diamond-choice apparatus. The trails were then stored in an air tight box for later bioassay in the same diamond-choice apparatus which was placed in the foraging trail between the nest and foraging arena. The trails from 1 major worker and from 3–5 major workers were collected and bioassayed in this manner. The trails were recorded as being laid by workers from the nest or from the arena. Well-established trails were obtained using a similar diamond-choice apparatus and typing paper but the termites from both the nest and arena were allowed to lay a trail for at least 2 hours.

The trails of a single major worker leaving the nest remains effective for 1 hour 30 min. while that of a worker leaving the arena is effective for 1 hour (Table 3).

The trail of 3–5 workers leaving the nest remains effective for 1 hour 45 min. while that of 3–5 workers leaving the arena is effective for 1 hour 15 min. (Table 3).

A well-established trail remains highly effective for 7 hours and is still attractive after 9 hours (Table 4).

Table 3. The longevity of natural trails from one major worker and 3–5 major workers

Time (h: m)	Iceaving nest				Iceaving area			
	No.	+	—	Significance level	No.	+	—	Significance level
				One worker trail				
0.45	44	32	12	$0.001 < p < 0.01$	47	34	13	$p < 0.01$
1.00	44	35	9	$p < 0.001$	41	30	11	$p < 0.01$
1.15	56	40	16	$0.001 < p < 0.01$	39	23	16	n.s
1.30	48	31	17	$0.01 < p < 0.05$	49	31	18	n.s
1.45	40	24	16	n.s	—	—	—	—
				3–5 worker trail				
1:10	57	46	11	$p < 0.001$	48	39	9	$p < 0.001$
1.15	57	43	14	$p < 0.001$	41	31	10	$0.001 < p < 0.01$
1.30	40	34	6	$p < 0.001$	39	23	16	n.s
1:45	70	46	24	$0.001 < p < 0.01$	50	28	22	n.s
2:00	48	27	21	n.s	—	—	—	—

Fig. 3:

The spontaneous sound producing activity of *G. morsitans morsitans* in LD 12:12 expressed as mean percentage of flies singing (ordinate; ± SE.) against time of day (abscissa). Solid circles indicate mean percent of female flies singing and solid squares indicate mean percent of male flies singing. Upper part of the figure shows the corresponding mean hourly temperatures (°C; open circles) and relative humidities (% R.H., open triangles).

Dark bands along the abscissa, show the scotophase period and the open band shows the photophase period.

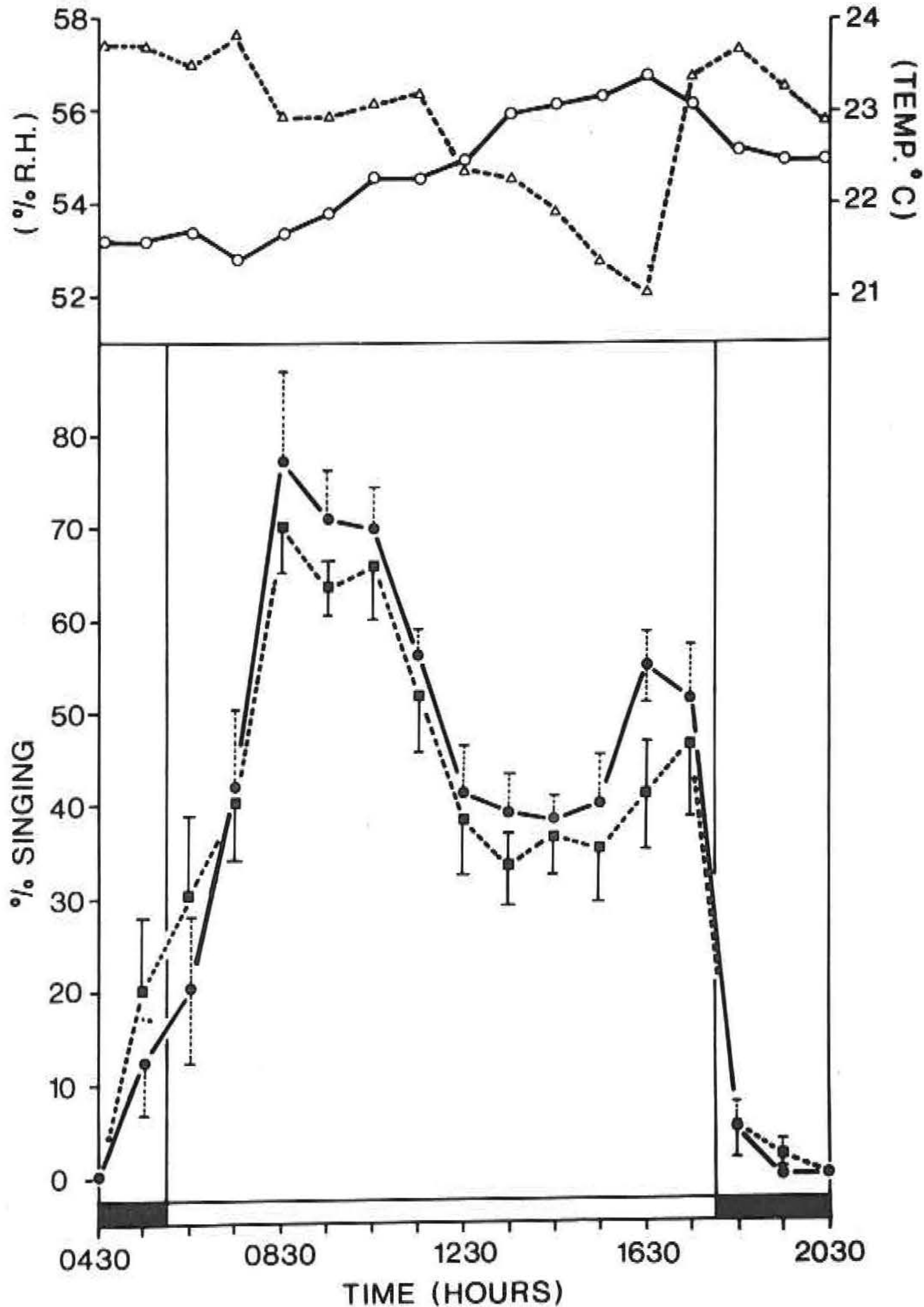


Table 4. Longevity of well-established trails of major workers

time (hr)	No.	+	—	significance level
7	38	36	2	$p<0.001$
8	45	37	8	p 0.001
9	36	27	9	$0.001<p<0.01$
10	23	10	13	n.s.
11	24	12	12	n.s.

The pattern of sound production by the tsetse fly *Glossina morsitans morsitans* Westwood

R. K. Saini

It has been known for a long time that various species of tsetse flies produce sounds variously described as singing, buzzing, squeaking, or pinging. These sounds are considered to be a means of communication and closely related to vital functions such as hunting, feeding, and mating. Although attempts have been made to record sounds relating to the above activities, the significance of these sounds is not yet fully understood. Studies were initiated to investigate the time and pattern of spontaneous sound production by the tsetse fly *Glossina morsitans morsitans* Westwood.

Newly emerged tsetse flies were fed and individual flies were then isolated in a hollow plastic vial (4.5 × 3.0 cm) sealed with dacron gauze at both ends. The vials were then placed in the laboratory in a 12 hour light: 12 hour dark cycle (LD 12:12) till the next day, when hourly observations for singing were made.

As shown in Fig. 3, a clear diel pattern of spontaneous sound producing activity emerged in LD 12;12: Singing activity was minimal during the scotophase. Less than 20% of the experimental flies produced sound half an hour before the onset of the photophase and less than 10% did so during the first one and half hour after the beginning of the scotophase. Sound production during the remaining part of the scotophase was either negligible or absent.

During the photophase, both male and female tsetse flies showed a clear "U"-shaped pattern of singing activity. The peak activity occurred during early morning; after which it started declining steadily till around mid-day when it reached the lowest level. In the late afternoon a second peak occurred at 1630 or 1730 hours. In total activity and duration the afternoon peak was less than half of the morning peak.

It should be noted that both males and females showed a similar "U"-shaped pattern of singing activity. Although the females sang more than the males during most of the photophase, the difference was not significant (X^2 (24) = 5.82). The males, both in the hour before and in the hour after "lights on", showed slightly more singing activity than the females, but the difference was not significant (X^2 (1) = 1.19 before "lights on" and x^2 (1) = 1.33 after "lights on").

It can be concluded that singing is modulated in the "U"-shaped pattern typical of other responses for *G. morsitans*. The rhythms of spontaneous flight activity, optokinetic responsiveness, olfactory responsiveness, resting preferences, defaecation frequency, and field biting activity, all show a "U"-shaped pattern of activity. The synchrony of singing with the above mentioned behavioural responses, indicates that sound production is related to the vital functions of hunting, feeding, and mating in a more significant way than has been assumed to be the case in the past.

The Tick Olfactometer

S. M. Waladde

In addition to the study of structure and electrophysiological response of olfactory receptors it is necessary to observe and quantify the gross responses of ticks to olfactory stimuli presented under controlled conditions. The need for this kind of behaviour studies led to the development of a modified Y-tube olfactometer (Fig. 4).

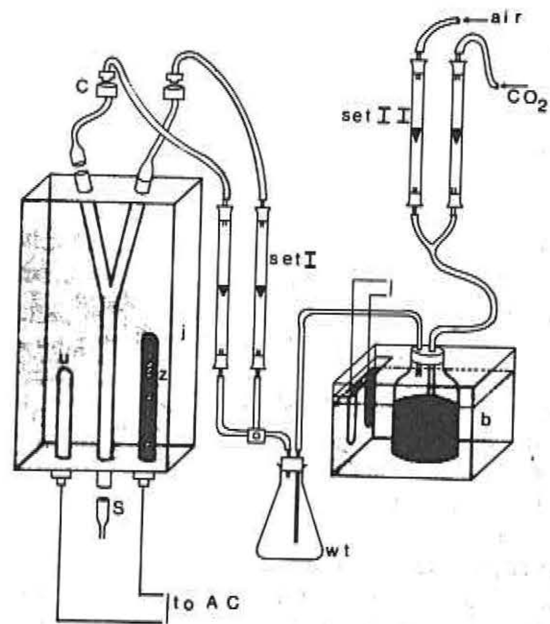


Figure 4. Diagram of tick olfactometer showing regions of the Y-tube and attachments: lower bottom (B) middle (M) left and right arms (L & R), specimen stage (S) stimulus or blank chambers (C) airflowmeters (set I and II), water jacket (j), thermostat (u) heater (z), water trap (wt) heated water bath (b) warm saturated sodium chloride (h).

Apart from the openings to the bottom and both arms of the Y-tube, the rest of the tube was enclosed in a transparent water jacket maintained at 37°C. This arrangement ensured that the walls of the Y-tube were maintained at a constant temperature. Each arm (11 mm i.d.) was connected to a stimulus or blank chamber which in turn was connected to an air flow-meter. This resulted in having two airflow meters (set I) which were regulated in such a way that the air current flowing into both sides of the olfactometer was uniform. The bottom opening was fitted with an outlet connected to a removable specimen stage which was used to introduce ticks into the olfactometer.

The air current flowing into each side of the olfactometer, at the rate of .65 litres per minute, was either ordinary air from a compressed air cylinder or a 4–5% CO_2 air mixture obtained by combing CO_2 and air from separate cylinders. Another pair of flowmeter (set II) was used in order to obtain the right proportions of the above mixture. Before any type of air current was allowed into the olfactometer it was humidified to 84% R.H. by bubbling it through a warm solution of saturated sodium chloride from which it went via a water trap and then into the flowmeters (set I) regulating through the olfactometer. In order to classify the activity and responses of ticks the Y-tube was divided into three regions, namely: lower bottom, middle, and the choice region (left and right arms) (Fig. 4). One tick was observed at a time for a period of 2 minutes during which its position and behaviour in the Y-tube were recorded every thirty seconds.

The first experiment was designed to compare the reaction of ticks subjected to a current of air only, with those subjected to a current of 4–5% CO_2 air mixture. In this comparison the ticks had no real choice because in either case both arms of the Y-tube were respectively subjected to the same conditions of air quality. From these tests it was noticed that there was no bias for either the left or right arm (Table 5). When air alone was flowing through the olfactometer, 65% of the ticks observed spent most of their time in the lower bottom region, 15% in the middle region while 20% moved into the left and right arms. However when the CO_2 air mixture was applied the distribution of ticks was as follows: 39% in lower bottom, 22% in middle and 39% in the arms.

A second experiment was designed to observe the responses of ticks to either a cattle wash or dog wash odour. These washes were obtained by scrubbing limited body areas of a steer or a dog with n-hexane and collecting most of the wash from the scrubbed areas. The debris in the wash was allowed to sediment and the supernatant was drawn off and concentrated in a rotary evaporator. Filter paper strips 10 × 40 mm were impregnated with 20 µl of either a cattle wash or dog wash concentrate and then used as odour sources when placed in one of the olfactometer stimulus chamber. The second chamber had a blank filter paper daubed with 20 µl of n-hexane (the solvent) which was allowed to evaporate before commencing the test. In this case ticks could make a choice between the Y-tube arm generating the cattle wash or dog wash odour and the blank arm. The odour carrier flowing into both sides of the olfactometer was the 4–5% CO_2 air mixture which had already been shown to boost tick activity. In order to avoid any bias, connections of the stimulus chamber and the blank chamber to the Y-tube were periodically alternated between the left and right arms.

Observations showed that ticks were strongly attracted to the cattle wash whereas the dog wash was not anywhere as attractive (Table 5). When the effect of CO_2 air mixture was compared with the effect of CO_2 air mixture + dog wash it appeared that the presence of the dog wash significantly caused more ticks to spend most of the time in the lower bottom region of the Y-tube (Table 5). Furthermore when the dog wash was being tested the majority of ticks initially made erratic movements, moved up into the middle region and then immediately returned to the lower bottom region. It seems that the dog wash may have a repellent component. No concrete conclusions have been reached yet but observations on these and other stimuli are still in progress. It is hoped that the olfactometer described here will be standardized for the purpose of studying tick behavioural responses to olfactory stimuli.

Table 5. showing prliminary treatments and the resultant distribution of ticks in the Y-tube at the end of a two minute observation period.

Treatments	Regions of Y-tube and tick numbers found			
	Lower bottom	Middle	Left arm	Right arm
Air alone	35	8	6*	5*
4–5% CO_2 air mixture	29***	16	18*	11*
			blank arm	odour arm
4–5% CO_2 air mixture + cattle wash	9	15	5**	51**
4–5% CO_2 air mixture + Dog wash	53***	16	7(**)	13(**)

* X^2 = .388 $P<0.05$ NS
** X^2 = 37.8 $P<0.001$ S
(**) X^2 = 1.8 $P<0.05$ NS
*** X^2 = 7.11 $P<0.01$ S

* (asterics) shows corresponding tick numbers compared and tested by X^2 test
NS=not significant
S=significant

BIOASSAY RESEARCH UNIT

Research Staff

Mr. G. Achieng (1976) Technical Assistant
Dr. T. Gebreyesus (1978) Research Scientist
Mr. L. Moreka (1976) Technical Assistant
Mr. B. N. Odero (1976) Principal Technician
Mr. E. N. Ole Sitaya (1979) Senior Technician

Services Provided by the Unit

T. Gebreyesus and
B. N. Odero

The Bioassay Research Unit was established in November 1977 with the main aim of providing routine bioassay services to the different Programmes and Units at the Centre. In addition to the Galleria wax test for juvenile hormone and the Musca test for ecdysones, which the Unit has provided on a routine basis since its establishment, it has extended its services during 1979 to include the following assays:

(1) Radio immune assay for ecdysones.
(2) Antifeeding test using the African armyworm (*Spodoptera exempta*) larvae.

The Unit has also established the following bioassays on an experimental basis:

(1) Fumigation tests for antijuvenile hormone activity using developing stages of grass-hoppers (*Gastromargus africanus*) and cotton stainers (*Dysdercus fasciatus*).
(2) Topital application of juvenile hormones on mealworm beetles (*Tenebrio molitor*).
(3) Dipping test for ecdysones using stem borers (*Chilo partellus*).
(4) Tick repellency test using adult *Rhipicephalus apendiculatus*.
(5) Larvicide test using larvae of *Aedes aegypti* mosquitoes.

In addition to providing routine bioassay services to the other Programmes and Units, the Bioassay Research Unit is also engaged in original research in developing new bioassays and in the screening of natural products from plant sources possessing insect activity in collaboration with other Research Units. The Unit maintains its own insectary.

Studies on antifeedants from plants

T. Gebreyesus

As part of a continuing study of natural products from plant sources for the control of pests, about forty five plants were collected in Western Kenya in October 1978. Most of these plants were indentified either by the Botany Department, University of Nairobi, or by the East Africa Herbarium. The project is carried out in collaboration with the Chemistry/Biochemistry Research Unit.

The identified plants were extracted with various organic solvents and the extracts tested for antifeeding activity using sixth instar African armyworm (*Spodoptera exempta*) larvae. The bioassay is carried out by cutting ten discs of young maize leaves (*Zea maize*) 1.5 cm in diameter, five of which are dipped in a solvent for about one second and the other five treated similarly with the plant extract in the same solvent and dried on a filter paper. The solvent-treated and extract-treated discs are then placed alternately in a petri dish and ten armyworm larvae, starved for at least two hours, are introduced. The amount of feeding is observed at half hourly intervals for two hours. A number of extracts showed moderate antifeeding activity whereas two showed significant activity. The chemicals responsible for the antifeeding activity in one of the plants have been isolated in pure form and their structure elucidation is proceeding. Work is in progress to isolate the antifeedant(s) in the other plant extract.

INSECT AND ANIMAL BREEDING UNIT

Research Staff

Mr. J. A. Atema (1975) Junior Technician
Mr. E. O. Awuoche (1973) Technical Assistant
Mr. H. Banda (1972) Technician
Mr. G. M. Birir (1978) Technical Assistant/Driver
Mr. A. S. Ikhunyalo (1971) Junior Technician
Mr. J. Kagoiya (1973) Technician
Mrs. R. Kariuki (1974) Subordinate Assistant
Mr. J. Ongudha (1973) Junior Technician
Mr. J. Wanyonje (1970) Senior Technician

Breeding of Tsetse flies

Breeding of *Glossina morsitans* using rabbits as host animals

A self sustaining colony of *G. morsitans* was maintained throughout 1979 at a temperature of 25°C.±1°C. and R.H.70%±10%. Tsetse emergence was very high during each month (Table 1). Total number of flies emerging was 71,279 males and 71,379 females. Mortality of adult females totalled 15,718 with the teneral mortality being very small. A total of 17,325 old females were removed from the colony and a total of 38,259 mated females added. Average number of females in the colony for each month is presented in the Table. A total of 167,108 pupae were collected: of these 27,113 were weighed showing an average weight of 29.1 milligrams per pupa. A total of 15,894 males; 17,588 females and 4,534 pupae were supplied for experimental use by ICIPE Scientists and other research workers.

Table 2 summarises the fecundity and the mortality of females of the *G. morsitans* colony.

Experimental Breeding of *Glossina pallidipes* using Rabbits as host animals.

A small colony of *G. pallidipes* was maintained at a temperature of 25°C±1°C. and R.H. 70%±10%. The main *G. morsitans* colony was used as a control.

Pupae collected from wild *G. pallidipes* caught in Lambwe Valley were remitted to Nairobi to start a clean colony. A first consignment of 711 pupae were received in January, 1979, starting emergence in February. Table 3 summarizes the colony performance during the year. The performance became poor during the later months because of inadequate facilities.

Table 4 summarizes the fecundity and the mortality of females of the *G. pallidipes* colony. *G. pallidipes* requires higher temperatures and relative humidities than does *G. morsitans* i.e. 28°C.±1°C.; 80%±10% respectively. If a suitable room could be obtained and the required conditions are maintained, *G. pallidipes* could thrive very well in the laboratory. A former animal room is now being converted into a *G. pallidipes* insectary to cater for this work. This work is being carried out in collaboration with Dr. R. S. Ochieng; at Mbita Point Field Station.

Table 1. Tsetse Colony performance: Species *G. morsitans*—Fed on Rabbits

	New Emergence		Mortality		Old ♀	Mated ♀	Av. Total ♀	Pupae Produced			Experimental Supplies		
Month	♂	♀	Virgin ♀	Mated ♀	Removed	Added	in Colony	Total Coll.	No. Weighed	Total Wt.	♂	♀	Pupae
Jan.	6575	6276	0	1200	2090	4200	5884	14795	1340	43.3676	2216	2910	1405
Feb.	6478	6318	0	0	775	4030	7002	13837	2942	87.0026	1279	1202	1079
Mar.	5541	5658	0	860	2362	3918	8044	15356	2925	81.5757	1502	2045	701
Apr.	6240	6381	0	536	1029	2550	6612	13112	2589	74.5980	1229	1070	400
May	5925	5876	0	2491	1132	2526	6927	15874	1657	47.2897	1211	1497	70
June	6539	6747	0	2517	947	3620	7105	13162	2113	61.1759	1265	1105	25
July	5837	5774	4	1465	1476	2710	6865	13126	2276	68.6040	1065	1295	135
Aug.	5313	5438	0	2410	816	2940	5614	11024	1899	54.3950	1088	1779	—
Sept.	4718	4675	0	2014	875	3110	5323	11925	2622	77.9723	1072	1055	350
Oct.	5867	5917	0	290	1657	3650	6139	14133	1315	38.7200	2163	1527	131
Nov.	5835	6017	0	685	1783	2140	6378	14473	2704	75.7390	1220	1910	181
Dec.	6411	6302	0	1250	2383	2865	4950	16291	2731	77.9080	584	175	57
Total	71279	71379	4	15718	17325	38259	—	167108	27113	788.3478	15894	17588	4543

Species: *G. morsitans* fed on Rabbits

Table 2. Summary of Fecundity and Mortality of Female Ttsetse Colony

Month	Total No. Pupae Collected	Average Pupa Production per ♀/Month	No. of Pupae Weighed	Total Wt. in Grams	Mean Wt. in Milligrams	Rate of ♀Mortality %
January	14795	2.5	1340	43.3676	32.4	0.7
February	13837	2.0	2942	89.0026	29.6	—
March	15356	1.9	2925	81.5757	28.0	0.3
April	13112	1.9	2589	74.5980	28.8	0.3
May	15874	2.3	1657	47.2897	28.5	1.2
June	13162	1.9	2113	61.1759	29.0	1.1
July	13126	1.9	2276	68.6040	30.1	0.7
August	11024	2.0	1899	54.3950	28.6	1.3
September	11925	2.2	2622	77.9723	29.7	1.2
October	14133	2.3	1315	38.7200	29.4	0.2
November	14473	2.3	2704	75.7399	28.0	0.3
December	16291	3.3	2731	77.9080	28.5	0.84

Table 3. Tsetse Colony Performance, Species *G. pallidipes*, Fed on Rabbit

Month	New Emergence		Mortality		Old ♀	Mated ♀	Av. Total ♀	Pupae Produced			Experimental Supplies		
	♂	♀	Virgin ♀	Mated ♀	Removed	Added	in Colony	Total Collected	No. Weighed	Total Wt.	♂S	♀S	Pupae
Jan.	—	—	—	—	—	—	—	—	—	—	—	—	—
Feb.	302	281	1	2	0	102	134	21	3	0.0933	0	0	0
Mar.	83	83	0	65	0	163	137	220	220	7.2380	0	0	0
Apr.	151	220	0	130	0	82	215	200	200	6.2524	0	0	0
May	190	172	0	38	0	229	159	208	146	6.1127	3	3	0
June	149	166	0	170	0	96	276	185	141	5.0214	5	5	0
July	240	188	0	68	0	80	204	184	184	5.9935	0	0	0
Aug.	136	160	0	95	0	128	215	203	185	6.0727	0	0	0
Sept.	139	131	0	77	0	0	222	223	223	7.0862	0	0	0
Oct.	225	236	0	0	0	130	166	164	52	1.7093	0	0	0
Nov.	57	76	0	64	0	30		131	93	2.9212	0	0	0
Dec.	40	42	0	0	0	0	246	71	49	1.4227	0	0	0
Total	1712	1755	1	709	0	1040	—	1810	1496	49.9034	8	8	0

Table 4. Summary of Fecundity and Mortality of Female Tsetse Colony; Species *G. pallidipes*, Fed on Rabbit

Month	Total No. Pupae Collected	Average Pupae Production per ♀/Month	No. of Pupae Weighed	Total Wt. in Grams	Mean Wt. in Milligrams	Rate of ♀Mortality %
January						
February	21	0.7	3	0.0933	31.1	0.2
March	220	1.6	220	7.2380	32.9	1.0
April	200	0.9	200	6.2524	29.1	2.0
May	208	1.3	146	6.1127	41.9	0.7
June	185	0.7	141	5.0214	35.6	2.9
July	204	1.0	184	5.9735	32.3	1.3
August	203	0.9	185	6.0727	32.8	1.4
September	223	1.0	223	7.0862	31.8	1.1
October	164	1.0	52	1.7093	32.9	—
November	131	0.05	93	2.9212	31.4	0.08
December	71	0.3	49	1.4227	29.0	—

LABORATORY OF INSECT PATHOLOGY

Research Staff

Mr. P. Amutalla (1978) Technical Assistant
Dr. G. P. Kaaya (1978) Postdoctoral Research Fellow
Dr. M. O. Odindo (1979) Postdoctoral Research Fellow
Dr. W. A. Otieno (1977) Research Scientist
Miss D. Sabwa (1979) Graduate Research Scholar

Mosquito Pathology:

Field Surveys of Naturally Occurring Pathogens of the Mosquito Vector, *Anopheles gambiae* complex

W. A. Otieno

A continuing survey of the Kenya Coast has been intensified during the year under review (1979) to determine the presence, abundance and importance of internal pathogens and external parasites of mosquitoes.

A total of 8600 mosquito larvae (*A gambiae*) representing all the instars were collected and examined. Protozoans, bacteria, fungi, virus, nematodes and epibionts were observed.

Of the diverse pathogens collected so far, the following offer greatest potential as Biocontrol agents:

. A fungus, *Coelomomyces* sp.
. A nematode, family Mermithidae

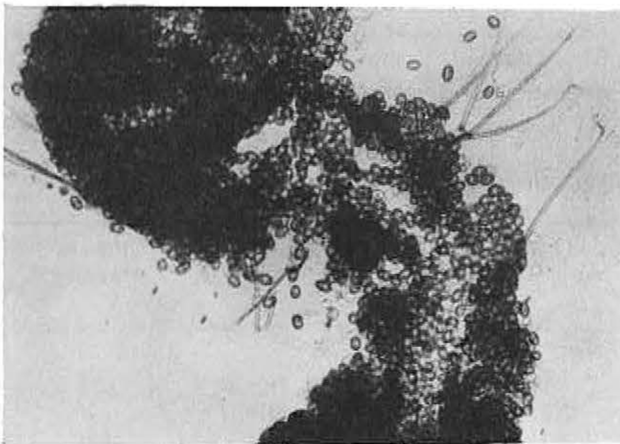


Figure 1. A fungus, *Coelomomyces* sp. in the mosquito larval host, *Anopheles gambiae*

Establishment of an *in vivo* laboratory culture of *Coelomomyces* sp. and its virulence to three mosquito species

W. A. Otieno and D. Sabwa

The genus *Coelomomyces* consists of approximately 40 described species of aquatic fungi, which recently have been shown to alternate obligately between copepods and mosquito hosts. Use of these fungi in control programmes has been hampered by an inadequate knowledge of the mechanisms leading to high mortalities in larval populations and the lack of methods for mass production of mosquito infective stages. Through laboratory and field studies, we are determining the parameters that will enable us to mass-produce and use this fungus in integrated mosquito control programmes.

Studies are in progress to determine the susceptibility of three important mosquito vectors: *Anopheles merus*, *Anopheles funestus* and *Culex fatigans* to the fungus, *Coelomomyces* sp.

Tsetse Pathology

W. A. Otieno, W. Snow and B. Turner

An active collaborative effort is underway to document the occurrence, abundance and distribution of the natural enemies (pathogens, parasites and predators) of tsetse flies as a prelude to in-depth experimental biological studies on the most promising pathogens for future development as biological control agents.

Results indicate that the following insect and microorganism (including nematodes) have parasitic and pathogenic associations with tsetse fly:

Nematode—(Nematoda: family Mermithidae)

A virus

Parasite: (Hymenoptera)

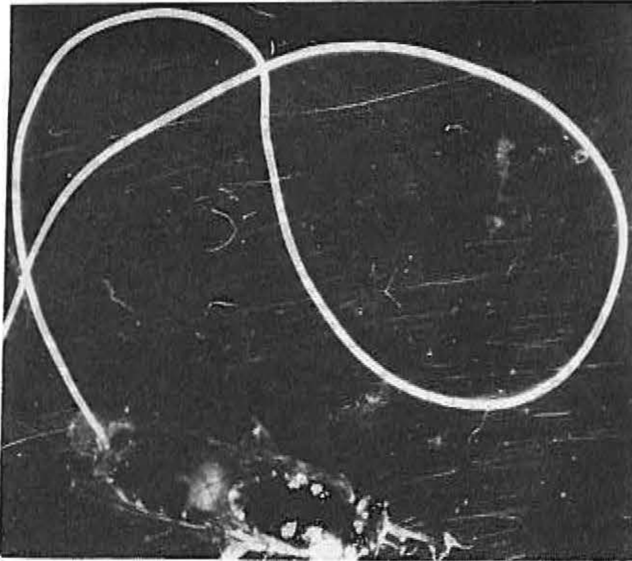


Figure 2. A nematode, Mermithidae from a female fly, *Glossina pallidepes*.

Pathology of sorghum shootfly (*Atherigona soccata*)

W. A. Otieno and A. G. L. Delobel

The following pathogens have been diagnosed and isolated.

. A fungus, *Entomophthora* sp.
. A bacteria, *Bacillus* sp.

Microorganisms from the Gut of larvae of the Sorghum and maize stem borer, *Chilo partellus*

W. A. Otieno, Z. T. Dabrowski and D. Sabwa.

A collaborative study of the gut microbial flora of *Chilo partellus* is being undertaken to understand the biological relationships between microorganisms and insects. The intestinal microflora in insects could be an important factor in the host plant-insect relationship. The insect microflora depends quantitatively and qualitatively on the physiological state of the food plants and also on the degree of bacteriophage activity of secondary metabolic substances (allelochemicals) contained in plant tissues. Allelochemicals are plant compounds responsible for antibiosis, adversely affecting larval survival and larval development on resistant plant cultivars. Thus the insect microflora may synthesize insect growth promoting substances, or may provide immunity to pathogens through inhibition of bacteria pathogenesis to the insect.

LIST OF PUBLICATIONS

This list has been compiled for the years 1972–1980. It contains papers published in scientific journals and/or in proceedings of conferences, and is by no means an exhaustive one. However, it represents most of the work done by ICIPE staff members. It is ICIPE's intention to update this list annually.

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